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ASSESSMENT OF PROXIMAL TUBULAR DYSFUNCTION

BY

ÅKE FREDRIKSSON

Uppsala 1975

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ASSESSMENT OF PROXIMAL TUBULAR DYSFUNCTION

An experimental study in the rat

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This thesis is based on the following papers. In the text they are referred to by their Roman numerals.

I Tubular transfer of glucose and o-iodo-hippurate in the normal rat kidney.
By Åke Fredriksson, Anders Engberg, Erik Persson and Hans Ulfendahl. 1975.
Scand J Urol Nephrol, Suppl. 26, 27-44.

II Tubular transfer of glucose and o-iodo-hippurate in experimental renal disease.
By Åke Fredriksson, Anders Engberg, Erik Persson and Hans Hamberg. 1975.
Scand J Urol Nephrol, Suppl. 26, 45-59.

III Effects of renal dysfunction on β_2-microglobulin metabolism.
By Åke Fredriksson and Per A. Peterson. 1975.
Scand J Urol Nephrol, Suppl. 26, 61-76.

IV Renal handling of β_2-microglobulin in experimental renal disease.
By Åke Fredriksson.
Scand J Clin Lab Invest. Accepted for publication.

V Influence of renal function on the urinary excretion of amylase. An experimental study in the rat.
By Åke Fredriksson and Göran Jacobson. 1974.
Scand J Clin Lab Invest 33, 313-321.

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LIST OF SYMBOLS

GFR = glomerular filtration rate, ml/min
$\dot{V}$ = urine flow rate, ml/min
T = tubular transfer, mg/min
Tm = tubular transfer above threshold, mg/min
Cp = concentration in plasma, mg/ml
Cu = concentration in urine, mg/ml
Ctf = concentration in the endproximal tubule segment, mg/ml
$\dot{V}$tf = volume flow rate in the endproximal tubule segment, ml/min
PEG = PEG 1000, polyethylene glycol, mol. weight 800-1000
G = glucose
OIH = o-iodo-hippurate
Prot = protein
PAH = para-amino-hippuric acid
φ = sieving factor, concentration in glomerular filtrate/concentration in plasma
Fmr = fractional metabolic rate, h^{-1}

Renal function is the sum of the functions of the large number of small morphological and functional units of the kidney, the nephrons. The various elements of renal function are performed by each nephron at different sites within each nephron. The proximal tubules make a very large contribution to the total kidney function. They perform a very energy-consuming process of reabsorption and secretion (Pitts, 1968). In renal disease the morphological changes are often situated in the proximal tubules and the interstitium surrounding them (Schirmeister, 1966). Despite these facts assessment of proximal tubule function in health and disease is very little utilized in clinical practise in contrast to measurement of glomerular filtration rate and concentrating ability of the distal parts of the nephron (Munck, Aurell & Giese, 1973). The conventional methods of estimating proximal tubule function are measurements of maximal tubule reabsorption of glucose and secretion of para-aminohippurates. However, these tests are complicated to perform and their interpretation is uncertain. Their interrelations with glomerular filtration rate, tubular reabsorption of salt and water, and plasma concentration are complex and still not completely understood (Morel & Rouffignac, 1973; Weiner, 1973).

The kidneys play a major role in the elimination of low molecular weight proteins from the body. Renal protein metabolism is changed in renal disease, and a tubular pattern of proteinuria is found as a qualitative sign of tubular dysfunction (Schultze & Heremans, 1966). Until now β_2-microglobulin seems to be the most suitable protein for estimation of proximal tubular dysfunction (Peterson, Evrin & Berggård, 1969; Ravnskov, 1973).

A reliable, quantitative method to estimate proximal tubular function is needed in clinical practise. This investigation has been designed to search for such a method. Renal handling of glucose, o-iodo-hippurate, β_2-microglobulin and amylase has been studied in clearance experiments on rats, with intact kidneys, and after induction of chemical, infectious or ischemic injury.

MATERIAL AND METHODS

Description and comments

Animals

Male albino rats of the Sprague-Dawley strain (Anticimex, Stockholm) were chosen as experimental animal, as they are inexpensive, easily available, fairly uniform in physiological behaviour, and therefore commonly used in investigations of renal physiology. Their weight at the induction of kidney damage was approximately 250 g. At the time of functional investigation their weight varied between about 250 and 500 g. The animals were fed on a standard diet (Ewos, Södertälje) and had free access to food and water until the start of the experiments.

Experimental groups

Controls. Untreated, apparently healthy animals were used as controls.

Aminonucleoside nephrosis was used as a model of glomerular injury resulting in increased permeability for large molecules like proteins (Oken, Cotes & Mende, 1972). A slight reduction of GFR in proportion to reduced blood flow has been reported and also slight changes in tubular morphology, partly due to increased endocytosis of protein (Nagle, Bulger, Striker & Benditt, 1972). It was induced by a single intravenous injection of 6-dimethyl-aminopurine-9-riboside (Sigma Chemical Co, St Louis, USA), 100 mg/kg body weight. The physiological investigations were performed after 10-14 days, when albuminuria was well established.

Sodium maleate injury was induced by infusion during 10 minutes of sodium maleate (Sigma Chemical Co) adjusted to pH 7.4 with NaOH. It is known to give enzymatic and morphological abnormolities mainly in the proximal tubules, with marked proximal tubular dysfunction, including tubular proteinuria (Mogielnicki, Waldmann & Strober, 1971). When 150 mg/kg body weight was given only tubular damage was seen and only little reduction of glomerular filtration rate. With 400 mg/kg body weight there was a considerable reduction in glomerular filtration rate probably due to blockage of tubules, but no changes in glomerular permeability and no morphological abnormalities of the glomeruli were observed (paper III). Physiological investigations were started one hour after the infusion of maleate.

Ischemic renal injury was achieved by temporary occlusion of the renal artery for 40 or 60 minutes. After 10 days there was an irreversible and stable kidney lesion, with marked damage of tubular epithelium, and interstitial inflammation, but intact glomerular structure (paper II and IV). Ischemic renal injury thus should be a model of tubular disease, and has implications on clinical situations with disturbances in renal circulation. Anaesthesia with intravenously injected Nembutal® (Abbott, Queensborough, Kent, England) was used, and body temperature was held at 37 ± 0.5°C with a thermostat -controlled heating pad. The importance of temperature variations during anaesthesia is often overlooked in experimental studies of renal ischemia. In pilot studies without the use of a heating pad a considerable drop in body temperature was noticed during anaesthesia with ether or Nembutal®, and this severely affected the degree of kidney damage. The external cooling of an exposed ischemic rat kidney is to be considered as well. Therefore the abdomen was closed during the ischemia period. The ischemia was applied only to the left kidneys, and the contralateral kidney left intact or removed after 8 weeks. Clearance studies were performed after 10-15 days (paper II) or 8 weeks (paper IV and V) in the former case, and 4 weeks after removal of the contralateral kidney (paper IV and V).

Pyelonephritis is known to be one of many causes of chronic interstitial nephritis, which is one of the most common pathological entities in renal pathology in man. A similar morphological picture was produced with the experimental pyelonephritis employed in this study. Pathological changes occur in the tubular structure, and are more marked and appear earlier than glomerular changes (Schirmeister, 1966). Interstitial changes correlate with alterations in renal function (Schainuck, Striker & Cutler, 1968). Thus a tubular dysfunction is to be expected in chronic pyelonephritis. Enterococci were used as they are known to give a long-standing infection with rather mild parenchymal damage (Guze, 1960). A modification of the method described by Shankel et al. was used (Shankel, Robson & Bricker, 1967). The kidney was punctured 100 times with a needle that was repeatedly dipped into a culture of enterococci, but no intravenous injection of bacteria was given. As this method implies considerable mechanical trauma, another method was introduced, where 0.15 ml of the enterococcal culture was injected by a stick through the renal parenchyma into the renal pelvis during 30 minutes of ureteral occlusion. The temporary occlusion was achieved by kinking the ureter up into a plastic tube with a silk thread. This procedure

allowed easy relief of the occlusion and seemed to cause no persistant damage to the ureter. With both methods disease was induced only in the left kidneys and the contralateral kidneys were left intact or removed at the same time.

Two to four months after induction of pyelonephritis functional studies were performed. At that time cultures from urine and renal parenchyma revealed more than 10^5 bacteria per ml urine or more than 10^4 bacteria per g tissue in about 60 % of the animals. Light microscopy revealed moderate chronic-subchronic interstitial nephritis. With the method of multiple kidney puncture, there was reduced weight of the diseased kidney in comparison with the intact kidney. When the intact kidney had been removed, there was considerable hypertrophy of the diseased kidney, and more tubular dilatation.

Normal single kidneys were subjected to physiological studies one hour after unilateral nephrectomy in normal rats. At that time the glomerular filtration rate of the remaining kidney was found to be 69 % of that for both kidneys before nephrectomy (Table I, p. 18). This accords with earlier studies, where a very rapid increase in glomerular filtration rate and a proportional increase in tubular reabsorption of salt and water are observed (Rous & Wakim, 1967).

Bilateral nephrectomy and bilateral ureteral ligation was employed in separate groups in metabolic studies with β_2-microglobulin. The experiments were started about half an hour after nephrectomy or ligation. As a complete ureteral obstruction may be difficult to establish in small animals, a double ligature was employed with kinking of the ureter between the two knots. A considerable glomerular filtration is shown to go on for several hours after bilateral ureteral ligation (Salomon & Lanza, 1962).

The experimental groups thus comprised models for the study of the effect of increased glomerular permeability (aminonucleoside-nephrosis), severe metabolic disturbance of tubular function (sodium maleate-injury), marked structural damage of the tubules (ischemic renal injury), and moderate tubular damage in combination with interstitial changes and possibly slight glomerular injury (pyelonephritis). In the groups with pyelonephritis and ischemic renal injury the function of the damaged kidneys was studied in an essentially normal environment, i.e. in unilateral renal disease with

the contralateral kidney intact. It was also studied after a reduction of the functioning kidney mass by removal of the intact kidney. The early effects of a reduction in kidney mass were investigated after unilateral nephrectomy in normal rats. The early effects of bilateral nephrectomy and bilateral ureteral ligation were included in the investigation of β_2-microglobulin metabolism.

The animals with ischemically injured left kidney and without the intact right kidney appeared to be anemic and in a slightly poor condition. Some of them died, probably due to uremia. All the other animals with ischemically injured kidneys, pyelonephritis or aminonucleoidnephrosis were in a good general condition. The animals with sodium maleate injury, bilateral or unilateral nephrectomy or bilateral ureteral ligation were used for only some hours after the induction of injury.

Substances used and analytical methods

Polyethylene glycol, molecular weight 800-1000 (Kebo, Stockholm), PEG 1000, was used to estimate glomerular filtration rate, GFR, (paper I, II, IV, V), since it has been reported to give more accurate values than inulin in the rat (Berglund, Engberg, Persson & Ulfendahl, 1969). In glucose studies 3H- and 14C-labelled PEG 1000 was used, and the activity was counted in a liquid scintillation counter. In studies with other labelled substances, unlabelled PEG 1000 was used, and it was analyzed turbidimetrically with a micromodification of the method used by Hydén (1955). Labelled PEG 1000 was given at 4 mg/hour, and unlabelled at 200 mg/hour. When labelled and unlabelled PEG 1000 were infused together they gave very similar values of glomerular filtration rate. In paper III endogenous creatinine-clearance was used for the estimation of GFR.

Glucose 50 % in water was used for estimations of proximal tubular reabsorptive capacity. It was determined with a glucose oxidase method (AB Kabi, Stockholm).

Ortho-iodo-hippurate sodium, OIH (Hippuran®, Mallinckrodt, New York, USA) was used to measure proximal tubular secretory capacity. It was mixed with ^{131}I- or ^{125}I-labelled OIH (Ab Atomenergi, Studsvik). For analyzis the samples were diluted in distilled water and the activity counted in a gamma-spectrometer. Labelled OIH does not give exactly the same clearance values as para-amino-hippuric acid and perhaps it is not 100 % freely

filterable (Bryan & Maher, 1973). However, there seems to be no direct measurements of the filterability of OIH in vivo. The differences in molecular weight between OIH and PAH has to be considered, as molar concentrations rather than weight per ml are of importance in for example threshold measurements. The analyzis of labelled OIH is more exact. On the whole labelled OIH parallels PAH, and may well serve as a test substance for proximal tubular function.

β_2-microglobulin was isolated from patients with tubular disease. After purification it was labelled with ^{125}I, and for analyzes the proteinbound activity was counted in a gamma-spectrometer. Human β_2-microglobulin was used as the rat homologue is as yet not available, but probably this is of little importance since β_2-microglobulin from several species are very similar and studies of endogenous retinol-binding protein and amylase gave corresponding results (see paper III, IV, V). The labelled protein was indistinguishable from its native counterpart by several chemical, physical-chemical and immunological criteria.

Amylase is a protein and the excretion rate thus ought to be dependant on renal handling of proteins. As it was found that only 10-20 µl of urine or plasma is required for analysis of amylase with the chromogenic method employed in this investigation (Phadebas Amylase Test®, Pharmacia, Uppsala), it was considered as suitable for a study of the renal handling of an endogenous, unlabelled protein.

Clearance experiments

The urinary excretion of glucose, OIH, β_2-microglobulin and amylase were studied in whole kidney clearance experiments. The animals were anaesthetized with Inactin®(Promonta, Hamburg), 120 mg/kg body weight injected intraperitoneally, which gives a stable anaesthesia. Tracheostomy was performed and the body temperature was held at $37 \pm 0.5^{\circ}C$ by a thermostat-controlled heating pad. The exogenous substances and additional saline were given as intravenous infusion. Blood sampling and blood pressure recording was performed through arterial catheters. Renal vein samples were taken by direct puncture through the inferior vena cava. Urine was collected under oil through ureteral catheters. The catheters were placed in the distal ureteral ends so that the ureteral peristalsis could overcome their resistance to flow. The drop in hydrostatic pressure over the catheter at maximal urine flow rate employed was approximately 6 cm

H_2O. Blood samples were taken in the middle of each clearance period, and the duration of the clearance period was varied in order to obtain an appropriate amount of urine. The amount of urine was determined by weighing.

Calculations and selection of proper variables in clearance studies of proximal tubular function

In calculations of glomerular filtration rate (GFR) and tubular transfer (Tm) of glucose and OIH the following conventional equations were used (for explanation of the symbols employed see page 3):

$$GFR = \dot{V} \cdot Cu/Cp\ (PEG) \tag{1}$$

$$Cu\ (G) \cdot \dot{V} = GFR \cdot Cp\ (G) - Tm\ (G) \tag{2}$$

$$Cu\ (OIH) \cdot \dot{V} = GFR \cdot Cp\ (OIH) + Tm\ (OIH) \tag{3}$$

Dividing equation (2) and (3) by GFR and rearranging gives

$$Tm\ (G)/GFR = Cp\ (G) - \frac{Cu\ (G)}{Cu/Cp\ (PEG)} \tag{4}$$

$$Tm\ (OIH)/GFR = \frac{Cu\ (OIH)}{Cu/Cp\ (PEG)} - Cp\ (OIH) \tag{5}$$

In clearance studies the tubular transfer of glucose and OIH cannot be directly measured. The calculations are based on measured values of urine flow rate ($\dot{V}$) and concentrations in plasma and urine of the test substances and the substance used for GFR measurements. The same measured values are used for the calculation of several physiological variables. These variables are then not mathematically independent which gives difficulties for the statistical analyzis. This is a common problem in physiology, but is often overlooked (Wesson, 1957; Engberg, 1969). If the variation of Tm with varying GFR is studied, GFR is used to calculate Tm. If Tm or Tm/GFR is studied at varying Cp, Cp is included in the calculation of Tm and Tm/GFR. In the present investigation the problem is solved by relating the independent part of Tm/GFR, i.e. $\frac{Cu}{Cu/Cp\ (PEG)}$, to Cp. Thereby the relation between Tm/GFR and Cp is indirectly established. $\frac{Cu}{Cu/Cp\ (PEG)}$ is the amount excreted per unit volume of glomerular filtrate (eq. 2-5).

In a study of proximal tubular transfer it would be of great interest to know Ctf, i.e. the concentration of the studied substance in the endproximal tubule segment, where the tubular transfer of glucose and OIH is supposed to cease. This would facilitate the analyzis of transport mechanisms. Thus it would be valuable to establish the relation of Ctf to variables measured in clearance studies. This can be done as follows.

The excretion of the substance should equal $Cu \cdot \dot{V}$, but also $Ctf \cdot \dot{V}tf$.

$$\dot{V}tf = \frac{GFR}{Ctf/Cp\ (PEG)}$$

Dividing $Cu \cdot \dot{V} = Ctf \cdot \dot{V}tf$ by GFR then gives

$$\frac{Cu}{Cu/Cp\ (PEG)} = \frac{Ctf}{Ctf/Cp\ (PEG)} \qquad (6)$$

Thus $\frac{Cu}{Cu/Cp\ (PEG}$ is also valuable for the discussion of tubular events leading to observed values for urinary excretion. However, it must be kept in mind that this calculation requires the assumption that no reabsorption or secretion occurs distal to the endproximal segment. Some transfer of glucose is reported to take place in the distal parts of the nephron (Frohnert, Höhmann, Zwiebel & Baumann, 1970). However, this transfer is small and would not affect the conclusion drawn. A reference substance to measure Ctf/Cp (PEG) would be of great value, but is not available.

The selection of proper variables to estimate protein excretion is discussed in paper V. There is the same problem of finding mathematically independent variables. The urinary excretion of plasma proteins depends upon glomerular filtration and tubular reabsorption, T (prot). The filtered amount depends upon plasma concentration of the protein, Cp (prot) and a sieving factor, φ(prot), that varies with molecular size (Lambert, Gassee & Askenasi, 1970). The excretion can be assumed to follow the equation

$$Cu\ (prot) \cdot \dot{V} = GFR \cdot Cp\ (prot) \cdot \varphi\ (prot) - T\ (prot)$$

If there is no threshold plasma concentration for urinary excretion, and protein excretion varies proportionally to Cp and GFR, the commonly employed clearance ratio, clearance for protein/GFR, seems to be a proper variable. If there is a threshold, the excreted amount per volume glomer-

ular filtrate $\frac{Cu\ (prot)}{Cu/Cp\ (PEG)}$, in relation to plasma concentration is proposed as the most suitable variable.

The renal extraction, i.e. relative renal arterio-venous difference, of β_2-microglobulin was measured and compared with that for PEG 1000. The renal extraction of a substance was calculated as

$$E_{(x)} = 1 - \frac{\text{renal vein concentration}}{\text{arterial concentration}}.$$

Turnover studies of β_2-microglobulin

The animals were prepared essentially as in clearance studies. The ^{125}I-labelled β_2-microglobulin was given as a single intraarterial injection. Arterial blood samples were taken and the experiments continued until a monoexponential plasma disappearance curve was obtained. The initial concentration was obtained by dividing the administered dose with the plasma volume, assumed to be 4 % of the body weight. Kinetic data were calculated according to Nosslin (1966), by using the peeling off technic. The equations used are given in Paper III. The method should be valid if the intravascular compartment is rapidly exchanging with the compartments of synthezis, catabolism and excretion (Waldmann & Strober, 1969). That seems to be the case for most plasma proteins. As in this investigation the life of the protein was very short compared to the half time for ^{125}I, no correction for diminished radioactivity had to be made. As it was a heterologous protein that was used, it was of course not in a metabolic steady state, but the turnover was not seen to be influenced by a large variation in plasma concentration, and studies with endogenous retinol-binding protein corroborates the data obtained (Östberg, Fredriksson, Rask, Peterson & Larson).

TUBULAR TRANSFER OF GLUCOSE AND O-IODO-HIPPURATE

Transfer maxima (Tm) of glucose and paraaminohippuric acid (PAH) at a plasma concentration over a threshold value have been used to characterize proximal tubule function. The first quantitative approach to the tubular handling of glucose was made by Hagedorn (1921). Later Ni and Rehberg (1930) concluded that the tubular reabsorption was limited by the concentration difference for glucose between tubular lumen and plasma. Shannon et al. (Shannon, Farber & Troast, 1944) presented results indicating trans-

fer maxima (Tm) for glucose. This theory was advocated by Smith (1951) and has since then been dominating in the nephrological literature. Still it is often recommended and referred to as a measure of functioning tubular mass.

However, in recent years there have been many clearance and micropuncture studies which make clear that a more dynamic attitude must be employed. The maximal tubular transfer of glucose and PAH are seen to vary with glomerular filtration rate (GFR), tubular flow rate and tubular reabsorption of fluid and sodium (Morel & Rouffignac, 1973; Weiner, 1973). The ratio Tm/GFR is often used instead of Tm. The existence of active, saturable processes is well established. The possible role of passive diffusion and bulk flow has also to be considered (Loeschke, Baumann, Renschler & Ullrich, 1969; Bryan & Maher, 1973).

In renal disease the GFR and tubular fluid reabsorption are often changed. If the transfer of glucose and PAH are to be used for assessment of proximal tubular dysfunction, the relation of the tubular transfer to GFR and tubular fluid reabsorption have to be known and the interrelationships between tubular dysfunction and GFR as well. Bricker et al. (Bricker, Klahr, Lubowitz & Slatopolsky, 1971) have made many important contributions to these problems. In unilateral pyelonephritis they found parallel decrease of GFR and several proximal tubular functions, including glucose and PAH. After removal of the intact kidney, there were non-parallel changes of GFR and tubular functions, a change of the so-called splay in the threshold region for glucose, and also a decrease in fractional fluid and sodium reabsorption in proximal tubules.

Present investigation

Tubular transfer of glucose and o-iodo-hippurate in the normal rat kidney (paper I)

A possible variation of the tubular transfer (Tm) and the ratio Tm/GFR with plasma concentration over the threshold was investigated. The plasma concentration was stepwise increased in each animal. The amount excreted per unit volume of glomerular filtrate, $\frac{Cu}{Cu/Cp\ (PEG)}$, was employed to make possible a valid statistical analyzis and to facilitate discussion of tubular events leading to urinary excretion (see Calculations, p. 10). A variation of Tm/GFR with plasma concentration was observed for both glucose and o-iodo-hippurate (OIH), with a tendency to increased reabsorption with increasing plasma concentrations. The variation was different between individual

animals or individual experiments. This difference was least at the "intersection points" of the individual regression lines, i.e. for glucose at the linear threshold, and for OIH at a plasma concentration of 1.0 mg/ml. From equation (6) and the observed values for $\frac{Cu}{Cu/Cp\ (PEG)}$ it is concluded that the tubular concentration of glucose and OIH increases more rapidly than does plasma concentration and probably exceeds plasma concentration even for glucose at higher values. This indicates that there is an increasing degree of diffusion with increasing plasma concentrations, which explains the tendency to increased reabsorption for both glucose and OIH. It is supposed that diffusion and fractional fluid reabsorption have their least influence on Tm and Tm/GFR at the intersection points, and the active transport therefore is best reflected at these points. It seems reasonable, that particularly the active transport is of value for assessment of impaired proximal tubular function.

Tubular transfer of glucose and o-iodo-hippurate in experimental renal disease (paper II)

The observations from paper I were employed in studies of pyelonephritis and ischemically injured kidneys. As for normal animals (paper I) there was a variation of Tm/GFR with plasma concentration in individual animals with a decreased fractional reabsorption for both glucose and OIH with increasing plasma concentration. In unilateral renal disease there were only small mean differences in Tm/GFR between diseased and intact kidneys, but statistically significant for OIH in one of the pyelonephritic and one of the ischemia groups. For comparison between the groups the "intersection points" observed in normal rats were employed. There was a much larger interindividual variation in the disease groups than for normal animals probably due to a varying degree of kidney damage. Mean values were lower for most of the diseased animals than for normal animals. In unilateral renal disease the intersection point values were usually decreased both for the diseased and the intact kidneys but for OIH they were lower in the diseased kidneys. It is concluded that the tubular transfer of glucose and OIH when measured at the intersection points is influenced both by damage to the tubular cells, and also by a functional adaptation in renal disease.

TUBULAR PROTEINURIA AND RENAL METABOLISM OF PROTEINS

Already Hippocrates observed the skimming of urine due to proteinuria, and connected it with kidney disease. Proteinuria is still a very important sign in detection of renal disease. Nowadays a more detailed analyzis of the quantity and kind of proteinuria is employed for diagnosis of the degree and localization of many renal diseases (Rennie, 1971). The proteins in urine originate partly from plasma and partly from the epithelium of the urinary tract (Berggård, 1970). Proteinuria is usually caused by an increased excretion of plasma proteins. Plasma proteins with a molecular weight above 90'000 are seen in urine only in trace amounts, due to a very low sieving factor. Exceptions from this are immunoglobulin A and G, that may be secreted from the uro-epithelium. The proteins with a molecular weight between approximately 40'000 and 90'000, like albumin, are excreted to some degree. For albumin the daily excretion is about 10 mgs (Berggård, 1970). In the glomerular filtrate the concentration of albumin is estimated to 1-2 mg/100 ml, corresponding to a filtration of 2-5 gms/24 hours. Thus a considerable tubular reabsorption must take place (Rennie, 1971). Low molecular weight proteins, with a molecular weight less than about 40'000, are easily filtered, but most of it is reabsorbed and very small amounts are excreted in the urine (Schultze & Heremans, 1966). The reabsorbed protein is degraded by the tubular cells and probably no intact protein is returned to plasma. In glomerular disease, there is often an increased glomerular permeability and thus an increased excretion of particularly albumin but also larger molecules. Another pattern, "tubular proteinuria", was demonstrated in chronic cadmium poisoning (Friberg, 1950) with marked tubular dysfunction. In this kind of proteinuria the excretion of low molecular weight proteins is largely increased, but also the excretion of albumin to some degree. The proportion of proteins of different size in urine has been used to differ between proteinuria of glomerular or tubular origin (Peterson et al, 1969).

β_2-microglobulin is a low molecular weight protein (molecular weight 11'800), first isolated by Berggård & Bearn (1968) in urine from patients with tubular proteinuria. It is likely to be the most suitable protein available in studies of tubular proteinuria in man, because it appears in plasma and urine essentially as a free monomer; its elimination is mainly performed by the kidneys and it seems almost freely filterable in the glomeruli. Normally it occurs in the urine only in trace amounts but in renal tubular dysfunction the excretion is increased up to 1'000-fold (Peterson et al, 1969).

The serum level correlates well with inulin clearance and is within narrow limits in humans without renal disease, with some exceptions in immunological and malignant diseases (Evrin & Wibell, 1972; Wibell, Evrin & Berggård, 1973). It can be accurately determined in urine and serum (Evrin, Peterson, Wide & Berggård, 1971). It occurs in several biological fluids, and several cell types, but its biological function is unknown. Since its amino acid sequence is homologous to the constant regions of the heavy and light chains of immunoglobulins and since it is a subunit of HL-A-antigens, it may well play a role in the immune system (Peterson, Cunningham, Berggård & Edelman, 1972; Peterson, Rask & Lindblom, 1974).

Amylase excretion in urine is used to diagnose pancreatic diseases. It has a molecular weight of about 50'000 (Thoma, Spradlin & Dugert, 1971), and the excretion rate reasonably should be influenced by the renal protein metabolisms. The influence of plasma concentration and glomerular protein filtration on the urinary amylase excretion has been pointed out by several investigators. In earlier studies on man and animals there seems to be no clear information on the role of tubular reabsorption (for ref. see paper V).

Present investigation

Effects of renal dysfunction on β_2-microglobulin metabolism (paper III)

The fractional metabolic rate (Fmr), renal extraction and urinary excretion was investigated in normal rats and in rats with different kinds of renal dysfunction. For normal rats the Fmr was 2.0/hour and the biological half-life 2.4 hours. The role of the kidney in β_2-microglobulin elimination was illustrated by a 6-fold decrease of Fmr after bilateral nephrectomy and an approximately 10 % lower concentration of β_2-microglobulin in renal vein than in arterial plasma.

In sodium maleate treated animals the Fmr seemed to vary with glomerular filtration rate, but the urinary excretion was largely increased because of the tubular dysfunction. In bilaterally ureter-ligated animals the Fmr was approximately the same as for normals. In sodium maleate treated, ureter-ligated animals the Fmr was moderately decreased. It is concluded that glomerular filtration and tubular reabsorption and degradation could continue despite disrupted urine flow, but if there was also tubular dysfunction, the proteinuric fraction could not be compensated for by tubular reabsorption and degradation.

This investigation confirms the view that β_2-microglobulin is eliminated mainly by the kidneys and by a process of glomerular filtration and tubular reabsorption and degradation. In tubular dysfunction there is a considerable urinary excretion.

Renal handling of β_2-microglobulin in experimental renal disease (paper IV)
The renal extraction and urinary excretion of β_2-microglobulin was studied in rats with ischemic renal injury, pyelonephritis and unilateral nephrectomy. The excretion was recorded as the ratio between clearance for β_2-microglobulin and glomerular filtration rate. The secretion of o-iodo-hippurate was measured for comparison.

The renal extraction was about 70 % of that for PEG 1000, except in the group with pyelonephritis and the intact kidney removed, where it was about 26 %. Thus the extraction of β_2-microglobulin seemed to parallel glomerular filtration and was somewhat lower than if the protein was freely filtered.

In unilateral renal disease the β_2-microglobulin excretion of the diseased kidneys was similar to that of the intact kidneys. A small but significant difference was noted only after ischemic renal injury. The same was found for o-iodo-hippurate. After removal of the intact kidneys the excretion of β_2-microglobulin was about 10-fold increased in pyelonephritic animals and 2- to 30-fold increased in animals with ischemic renal injury. One hour after unilateral nephrectomy in normal rats the excretion increased about 50 %. For the secretion of o-iodo-hippurate there was no consistent change.

It is concluded that glomerular filtration is a main step in the renal catabolism of β_2-microglobulin, and that its urinary excretion is influenced not only by tubular cell lesions, but, perhaps to an even greater degree, by the functional adaptation to a reduction in functioning kidney mass.

β_2-microglobulin turnover in unilaterally nephrectomized rats
The turnover of β_2-microglobulin was investigated in 5 unilaterally nephrectomized rats. The β_2-microglobulin was given one hour after the nephrectomy. The results are given in Table I. The turnover rate was about the same as for normal rats (paper III). The glomerular filtration rate was determined in another group of animals. One hour after nephrectomy it was

Table I. Turnover of β_2-microglobulin and glomerular filtration rate in normal and unilaterally nephrectomized rats

Group	Glomerular filtration rate (GFR) ml/min / 100 g BW	Fractional metabolic rate (Fmr) 1/h	Biological half-life (BHL) h	Intravascular distribution (% IV) %
Normal n = 7	0.90 ± 0.08	2.01 ± 0.20	2.4 ± 0.3	27.5 ± 4.9
Unilaterally nephrectomized n = 5	0.62 ± 0.11	2.25 ± 0.51	2.5 ± 0.2	32.8 ± 5.5

All values are given as mean ± SD

69 % of that of both kidneys before nephrectomy. This rapid increase in GFR in the single remaining kidney can partly explain the normal fractional metabolic rate, but some additional explanation seems necessary. A slightly increased glomerular permeability is possible, although this could not be detected (paper IV).

Influence of renal function on the urinary excretion of amylase (paper V)
The urinary excretion of endogenous amylase was studied in normal rats and in rats with different kinds of induced kidney lesions. In normal rats the amylase excretion seemed to increase proportionally with increased plasma concentration of amylase and glomerular filtration rate. The ratio between amylase clearance and glomerular filtration rate varied only slightly with body weight and was independent of urine flow rate. Both increased glomerular permeability to proteins in aminonucleoside nephrosis and tubular dysfunction after sodium maleate treatment augmented this ratio. In unilateral pyelonephritis and unilateral ischemic renal injury this ratio remained unchanged, but after removal of the intact kidney the ratio increased considerably. After unilateral nephrectomy in normal rats the ratio was unchanged. The results are illustrated in Fig. 1.

It is concluded, that normally there is a considerable tubular reabsorption of filtered amylase, estimated to be 50-70 %. The sieving factor for amylase is roughly estimated to be 2.5 - 4.5 %.

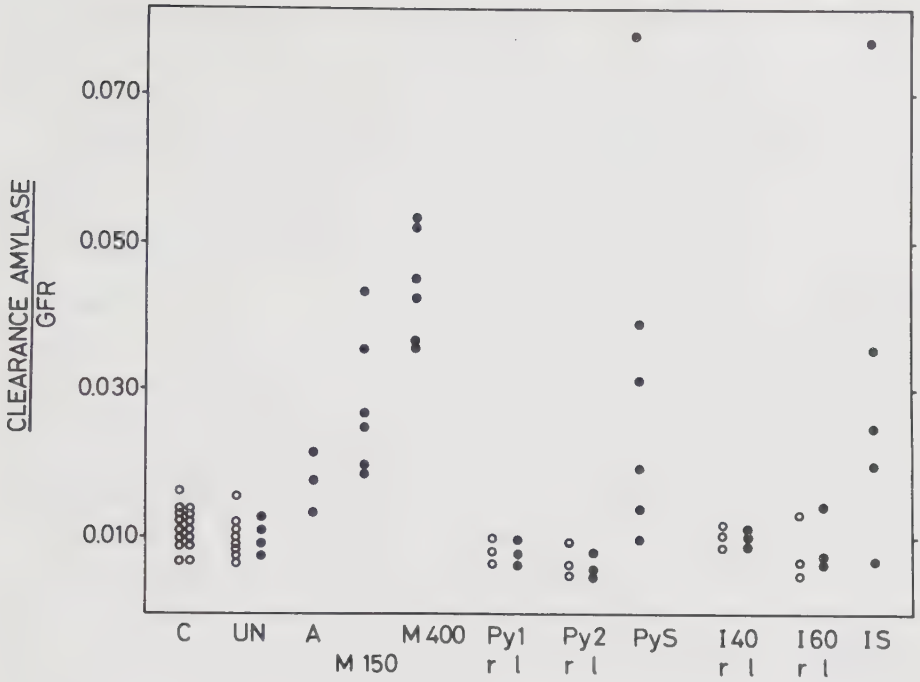

Fig. 1. The ratio clearance amylase/GFR for the experimental groups. The points represent mean values for each kidney. In the group NS o = right and left kidneys before, and ● = left kidneys after removal of the right kidney. In the other g roups o = kidneys without induced damage and ● = kidneys with induced damage. r = right kidneys, l = left kidneys.

It is also concluded that the amylase excretion is considerably influenced by a reduction of the functioning kidney mass. In this respect these results with endogenous amylase support the corresponding observations with human β_2-microglobulin in paper IV.

GENERAL DISCUSSION AND CONCLUSIONS

The purpose of this investigation was to study the possibility of making a valid assessment of proximal tubular dysfunction in renal disease. Despite the fact that the proximal tubules are often affected in renal disease, the available methods of measuring proximal tubular function are rarely employed in clinical practise. The reason for this is that these methods are often complicated to perform and also difficult to interpret (Munck et al, 1973).

As the proximal tubules are not available for direct study in clinical practise, indirect measurements from clearance studies have to be relied upon. This sometimes makes the mathematical treatment of observed data unreliable as the same observed values are often used in the calculation of several variables (see p. 10). In this study the amount of glucose or o-iodohippurate (OIH) excreted per unit volume of glomerular filtrate, $\frac{Cu}{Cu/Cp\ (PEG)}$ was chosen as an independent variable in correlations with plasma concentration.

A reliable measure of GFR is necessary, particularly if the tubular transfer is measured at high plasma concentration, where it is small in comparison with the filtered load. Unfortunately the reliability of conventional methods for GFR measurements in renal disease is insufficiently established (Levinsky & Levy, 1973). The distal parts of the nephron may participate to some degree in the tubular transfer of the substances used to measure proximal tubular function (Frohnert et al, 1970), but this transport is found to be small. To obtain more exact and detailed information on proximal tubular function, it is necessary to employ micropuncture techniques which are also necessary for the understanding of clearance data. As in this study the object was to find a clinical application, the clearance technique was chosen.

To assess the tubular dysfunction in renal disease, it is necessary to consider the normal physiological variation. The method for measurement of glucose and OIH transfer suggested in paper I was the use of the so called "intersection points". This method seems more adequate than Tm or Tm/GFR, as it takes into consideration the variations with plasma concentration, and the interindividual variation, in addition to the influence of GFR. Although it might decrease the influence of alterations in fractional fluid reabsorption, this influence cannot be abolished, as fractional fluid reabsorption cannot be measured in clearance studies. A disadvantage of the use of glucose and OIH is that osmotic diuresis is induced and thereby fractional fluid reabsorption in proximal tubules is probably reduced (Seely & Dirks, 1969).

For the assessment of protein excretion the ratio between clearance of protein and GFR is commonly employed, as it takes into consideration variations with plasma concentration and GFR. This seems valid if there is no threshold plasma concentration, and if there are no large variations with

body weight or urine flow rate, as is found for amylase (paper V). If there is a threshold, the amount excreted per unit volume of glomerular filtrate seems more valid. However, to estimate the tubular reabsorption of a protein, it is necessary to know the glomerular sieving factor for the calculation of the filtered load. An exact value for this factor is rarely available, as micropuncture data for proteins are scarce.

In metabolic impairment of tubular function as caused by sodium maleate in this study, there is a considerable increase of the excretion of β_2-microglobulin (paper III) as of other low molecular weight proteins. The excretion of amylase is also increased (paper V). There is also abnormal excretion of glucose as well as other signs of proximal tubular dysfunction (paper III). However, in ischemic renal injury, where there are marked structural lesions in the tubules, there are only small differences in OIH transfer (paper II) and β_2-microglobulin excretion (paper IV), between diseased and intact kidneys, in unilateral renal disease. The reason for this might be that in healed ischemic lesion the quality of renal tubular function remains normal, and that the diminished functional capacity is balanced by a reduced GFR (Mason, Takabatake, Olbricht & Thurau, 1974). There may also be other mechanisms of functional adaptation. In metabolic impairment such regulatory mechanisms could be put out of function.

In uremia an increased protein excretion in relation to GFR is seen for several proteins in man (Ravnskov, 1973; Wibell, 1974) and changes in glucose reabsorption are also observed (Bricker et al, 1971). Similar observations are made in this study. When the functioning kidney mass is reduced by removal of the intact kidney in unilateral renal disease, a sometimes very large increase is seen in the excretion of β_2-microglobulin and amylase, even in pyelonephritis, with less tubular injury (paper IV and V). A decrease in glucose reabsorption is also observed. Furthermore, for glucose and OIH the values for the intact kidneys in unilateral renal disease were also changed and in the same direction as for the diseased kidneys (paper II). These facts are taken as evidence, that functional adaptation in renal disease influences the assessment of proximal tubular dysfunction. This adaptation may mask tubular dysfunction or may directly influence tubular transport. The mechanisms involved may be tubulo-glomerular feedback, increased GFR per nephron, altered glomerular permeability, changed fractional fluid reabsorption and hormonal or circulatory changes (Seldin, Carter & Rector, 1971).

Although the relevance of experimental investigations to clinical practise is limited, some clinical implications from this study should be noted. Of the substances used in this study OIH may be equally as sensitive as β_2-microglobulin to reduced tubular capacity without profound metabolic disturbance, but less influenced by the functional adaptation in renal disease. However, an exact measurement of the OIH-transfer is complicated (paper I and II) and therefore β_2-microglobulin is probably preferable, but it would be valid as a measure of proximal tubular dysfunction only when GFR is normal or only moderately reduced (paper IV). β_2-microglobulin is sensitive to metabolic tubular disturbances (paper III). It could be of value to compare the serum concentration of β_2-microglobulin with creatinine concentration in urinary obstruction, as the fractional metabolic rate of β_2-microglobulin is unchanged in acute urinary obstruction (paper III). The β_2-microglobulin excretion would be valuable as a reference when determing the urinary excretion of enzymes or hormones of protein nature (paper V).

For other types of renal disease than those included in this study, the pattern of proximal tubular function may be different. However, the aspects of proximal tubular disease included in this study should reasonably have some bearing on measurements of proximal tubular dysfunction in all kinds of proximal tubular lesions. Other proximal tubular functions used for measurement, should reasonably be more or less influenced by the same factors as are the tubular transfer of glucose, OIH and β_2-microglobulin.

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TUBULAR TRANSFER OF GLUCOSE AND O-IODO-HIPPURATE IN THE NORMAL RAT KIDNEY

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ABSTRACT

The tubular transfer of glucose and labelled o-iodo-hippurate (OIH) above threshold (Tm) was studied, as a function of plasma concentration, in clearance experiments on normal rats. Glomerular filtration rate (GFR) was estimated as clearance of polyethylene glycol, molecular weight 800-1000 (PEG 1000). Plasma concentration of glucose and OIH was varied over a wide range in each animal. The amount of glucose or OIH excreted per unit volume of glomerular filtrate was correlated to plasma concentration and the relation between Tm/GFR and plasma concentration was calculated. A linear correlation was found for each animal.

Tm/GFR was observed to vary with plasma concentration. There was a decreased fractional excretion of both glucose and OIH with increasing plasma concentration in most animals. However, a variation between individual animals or experiments was found. This variation was least at common intersection points of the regression lines of the individual animals in the glucose- and OIH-group, respectively. The intersection point was near the linear threshold for glucose and at a plasma concentration of 1.0 mg/ml for OIH. It is suggested that active tubular transfer is best reflected at these points on the titration curves, and that these points therefore should be suitable to use in studies of impaired tubular function. From the amount excreted per unit volume of glomerular filtrate the endproximal tubular concentration was estimated. It seems reasonable that the latter increases much more rapidly than plasma concentration, and that at high levels diffusion out of the tubules is important for the net transfer of glucose and OIH.

Key words: Kidney function; proximal tubules; clearance experiments; glucose and o-iodo-hippurate transport; kidney function tests.

Transfer maxima for glucose , Tm(G) , and para-aminohippuric acid, Tm(PAH) , has been used to estimate the functioning proximal tubular mass (Smith 1951 , deWardener 1973). The existence of active, saturable transport mechanisms seems well established (Bayer,von Conta,Haeberle,Deetjen 1973 , Burg & Orloff 1969 , Chesney,Sacktor,Rowen 1973,Loeschke & Baumann1969,Tune & Burg 1971) and has been employed to explain a Tm-limited transfer. However, in numerous investigations with varying glomerular filtration rates (GFR) , Tm has been observed to vary with GFR , and therefore Tm/GFR has been used to characterize proximal tubular transfer (Handley,Sigafoor ,La Forge 1949 , Engberg 1969,van Liew ,Deetje Boylan 1967,Kwong , Bennett 1974). Furthermore, with micropuncture techniques it has been found that Tm(PAH) is flow dependent (Deetjen , Sonnenberg 1965 , Baines 1971). Studies of the dynamics of glucose and PAH transfer have revealed a coupling to sodium reabsorption (Ullrich , Rumrich,Klöss 1974,Vogel,Lauterbach Kröger 1965). In conditions with a decreased fractional reabsorption of sodium in the proximal tubules , as occurs during saline volume expansion , the reabsorption rate of glucose is decreased (Baines 1971 , Robson,Srivastava , Bricker 1968 , Kurtzman , White,Rogers , Flynn 1972) so that Tm/GFR is also decreased. During haemorrhage , aortic constriction and inferior vena cava constriction the fractional reabsorption of sodium is increased , and then Tm/GFR for glucose is also increased (Schultz & Berger 1973,Kurtzman et al. 1972). Thus , there seems to exist a dependance of glucose and PAH-transfer on sodium and fluid volume reabsorption The possibility of passive transfer diffusion across the tubular wall has been demonstrated by Loeschke et al. (Loeschke , Baumann,Renschler,Ullrich 1969). Diffusion may take place in either direction , and may contribute to the tubular transfer in different ways at varying tubular and plasma concentrations.

For all the above-mentioned mechanisms the net tubular transfer of glucose and PAH would be influenced by the tubular and plasma concentrations of glucose and PAH. From studies on isolated kidneys it has been suggested that the reabsorption of glucose is correlated positively with GFR and negatively with the concentration of glucose in the perfusate (Bowman & Maack 1972). Bryan & Maher (1973) have suggested that the transport of o-iodo-hippurate (OIH) was dependent on plasma concentration. It thus seems essential to investigate the relation of Tm/GF to plasma concentration of glucose and PAH . Van Liew et al (van Liew ,Deetjen , Boylan 1967) found a constant Tm/GFR for glucose. However , most studies are made on whole groups of animals , without regard to possible individual changes. It has also to be considered that if Tm/GFR and plasma concentrations are correlated , they are not mathematically independent , as the plasma concentration is used to calculate Tm/GFR.

The purpose of this investigation was to study Tm/GFR as a function of plasma concentration for glucose and OIH. The experiments were designed to avoid changes in extracellular volume. To include individual variation, plasma concentration was varied over a wide range in each animal, and the excretion per unit volume of glomerular filtrate was chosen as a variable that is mathematically independent of plasma concentration. The relation of Tm/GFR to plasma concentration could then be calculated.

Tm/GFR was found to vary with plasma concentration. Furthermore it was found that the calculated regression lines for the correlation between the excreted amount per unit volume of glomerular filtrate and plasma concentration had common intersection points for glucose and OIH, respectively. From the regression lines it can be speculated that endproximal tubular concentration of glucose and OIH will be very high at high plasma concentrations and that diffusion out from the tubules occurs.

MATERIAL AND METHODS

Animals

The experiments were performed on 12 male albino rats of the Sprague Dawley strain (Anticimex, Stockholm) weighing between 265 and 490 grams. They were fed on a standard diet (Ewos, Södertälje) and had free access to food and water until start of the experiments. 6 animals were used for glucose studies, and 6 for o-iodo-hippurate (OIH) studies.

Clearance experiments

The rats were anaesthetized by Inactin® (Promonta, Hamburg, 120 mg/kg B.W.) injected intraperitoneally. Tracheostomy was performed and the body temperature was held constant at $37.0 \pm 0.5^{\circ}$ C by a thermostat -controlled heating pad. Polypropylene catheters (PP 10 or 50) were inserted into a jugular vein for infusions and into a common carotid or femoral artery for blood sampling and blood pressure recording. The distal ureteral ends were catheterized with polyethylene catheters (PE10), and the urine was collected under oil. The amount of urine was determined by weighing.

To achieve different plasma concentrations of glucose and OIH above the renal threshold, the rate of intravenous infusion was increased at regular intervals in each rat. At each rate an equilibration period of 45 minutes was used for glucose and 30 minutes for OIH. One clearance period was performed for each infusion rate and 3-6 periods per animal (Table I). The duration of the period was varied in order

Table I. Regression lines of the amount excreted per unit volume of glomerular filtrate as a function of plasma concentration. Values are given for each kidney.

Kidney	Clearance periods, n	Plasma concentration mg/ml range	Slope, b	Correlation coefficient, r
Glucose				
A right	4	4.2 - 9.5	0.94	0.99
left	4		0.87	0.99
B right	4	4.6 - 9.2	1.01	0.99
C right	4	4.7 - 9.3	0.62	0.98
left	4		0.66	0.98
D right	5	4.6 - 9.0	0.68	0.99
left	5		0.82	0.99
E right	5	5.2 - 29.2	0.52	1.00
left	5		0.52	1.00
F right	6	4.7 - 7.4	1.31	0.98
left	6		1.47	0.96
O-iodo-hippurate				
G right	3	0.48 - 1.28	0.71	0.99
H right	3	0.46 - 0.97	0.25	0.95
left	4		0.55	0.98
I right	4	0.38 - 1.49	0.56	1.00
left	6		0.78	0.99
K right	6	0.39 - 1.64	0.86	0.99
left	6		0.88	0.97
L right	5	0.30 - 1.76	1.09	0.99
left	5		1.06	1.00
M right	6	0.60 - 2.90	0.53	0.99
left	6		0.42	1.00

to obtain an appropriate amount of urine (≥0.2 ml). Arterial blood samples (0.3 ml) were withdrawn in the middle of each period. GFR was estimated as clearance for PEG 1000 (polyethylene glycol, MW 800-1000)(Berglund, Engberg, Persson, Ulfendahl 1969) given as a separate constant-rate infusion. In the glucose group additional saline was given corresponding to the urinary sodium loss. Body weight was measured before and after the experiments.

The following infusions were given:

Glucose experiments:

Glucose 50 % in water	2.0 - 5.0 ml/h
^{14}C-PEG 1000[a], 0.25 μCi/mg, 4 mg/ml in saline	0.8 - 1.0 ml/h

O-iodo-hippurate experiments:

Ortho-I-hippurate sodium[b] 150 mg/ml, Ortho-^{125}I-hippurate sodium[c] 5 μCi/ml in saline	1.0 - 2.7 ml/h
PEG 1000[d] 200 mg/ml, in saline	1.0 ml/h

Analytical methods

Glucose was determined employing a glucose oxidase method (AB Kabi, Stockholm)

For analysis of ^{125}I, 50 μl of urine or plasma was diluted in 1 ml of distilled water and counted in a gamma-spectrometer (Autowell).

For analysis of ^{14}C, 100 μl of urine or plasma was suspended in 10 ml of liquid scintillation fluid[e], and counted in a Packard Tri-carb liquid scintillation counter.

PEG 1000 was analyzed turbidimetrically with a micromodification of the method used by Hydén (1955). Protein and sulphate were precipitated with barium hydroxide and zincsulphate. Exactly five minutes after addition of trichloroacetic acid the turbidity was determined with a spectrophotometer (Beckman Spinco) at 400 nm.

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a NEN, Frankfurt
b Mallinckrodt, New York
c AB Atomenergi, Studsvik
d Kebo, Stockholm
e Instagel, Packard

Calculations

The following abbreviations are used:

GFR = glomerular filtration rate, ml/min
$\dot{V}$ = urine flow rate, ml/min
Tm = tubular transfer above threshold, mg/min
Cp = concentration in plasma, mg/ml
Cu = concentration in urine, mg/ml
Ctf = concentration in the endproximal tubule segment, mg/ml
$\dot{V}tf$ = volume flow rate in the endproximal tubule segment, ml/min
(PEG) = PEG 1000, polyethylene glycol, molecular weight 800-1000
(G) = glucose
(OIH) = o-iodo-hippurate sodium

The following conventional equations were used:

$$GFR = \dot{V} \cdot Cu/Cp\ (PEG) \qquad (1)$$

Glucose excretion rate

$$Cu(G) \cdot \dot{V} = GFR \cdot Cp(G) - Tm(G) \qquad (2)$$

O-iodo-hippurate excretion rate

$$Cu(OIH) \cdot \dot{V} = GFR \cdot Cp\ (OIH) + Tm(OIH) \qquad (3)$$

Dividing equations (2) and (3) by GFR and rearranging gives

$$Tm(G)/GFR = Cp(G) - \frac{Cu(G)}{Cu/Cp(PEG)} \qquad (4)$$

$$Tm(OIH)/GFR = \frac{Cu(OIH)}{Cu/Cp(PEG} - Cp\ (OIH) \qquad (5)$$

Cu(G) or Cu(OIH) divided by Cu/Cp(PEG) thus is the amount excreted per unit volume of glomerular filtrate.

To derive an expression of Ctf it is assumed that no reabsorption of glucose occurs distal to the endproximal tubule segment. Then the amount of glucose excreted $Cu(G) \cdot \dot{V} = Ctf(G) \cdot \dot{V}tf$.

Dividing by GFR gives

$$\frac{Cu(G)}{Cu/Cp(PEG)} = \frac{Ctf(G)}{Ctf/Cp\ (PEG)} \qquad (6)$$

The same relationship can be shown for OIH.

Statistical methods consisting of regression analysis, comparison of the slopes of several regression lines, paired t-test and confidence limits were made according to Snedecor & Cochran (1967). Only observations well above the threshold value were included. Of a total number of 111 observations 5 were excluded, as they differed greatly from the majority of observations.

RESULTS

Data obtained from clearance studies and recorded as a function of plasma concentration (Cp) during one experiment with glucose and one with OIH are illustrated in Fig. 1 a and b. Urinary concentrations, Cu(G) and Cu(OIH), reached plateau values soon after the thresholds were passed. Urinary concentration of glucose and OIH were estimated to be of similar magnitude, about 2-400 mOsm/l. Thus when excretion rate of glucose and OIH increased this occurred as a consequence of an increased urine flow. GFR was seen to decrease at high Cp for glucose but not for OIH. The tubular transfer, Tm, was found to vary with Cp in most experiments. The ranges of plasma concentration are given in Table 1. The range of body weight changes were -1 to + 2 % for the glucose group and -1 to -5 % for the OIH group.

The amount of glucose or OIH excreted per unit volume of GFR, $\frac{Cu}{Cu/Cp(PEG)}$ (see calculations, eq. 4 and 5), was correlated to Cp for individual rats. A linear correlation such as shown in Fig. 1 was found in every experiment ($p < 0.001$). The slopes of the calculated regression lines (Fig 2 and Tab. 1) were significantly different between individual rats and in most cases differed significantly from 1.0 ($p < 0.001$). Most of the slopes were below 1.0 with a few higher than 1.0. This would mean that Tm/GFR varied with varying plasma concentration (Fig 3) and that this variation was different in different animals. As most of the slopes were lower than 1.0, Tm(OIH)/GFR decreased and Tm(G)/GFR increased with increasing Cp for 9 of 12 animals (Table 1, Fig. 2 and 3). In fact, at the highest plasma concentrations of OIH, the Tm(OIH)/GFR was negative, which would mean that net tubular transfer was reabsorption. The slopes for the two kidneys in each rat did not differ significantly. Mean differences between $\frac{Cu}{Cu/Cp(PEG)}$ for left and right kidneys were 0.08 ± 0.40 for glucose and 0.06 ± 0.08 for OIH (confidence limits, $p = 0.95$).

The regression lines (Fig 2) appear to intersect each other at a common point near

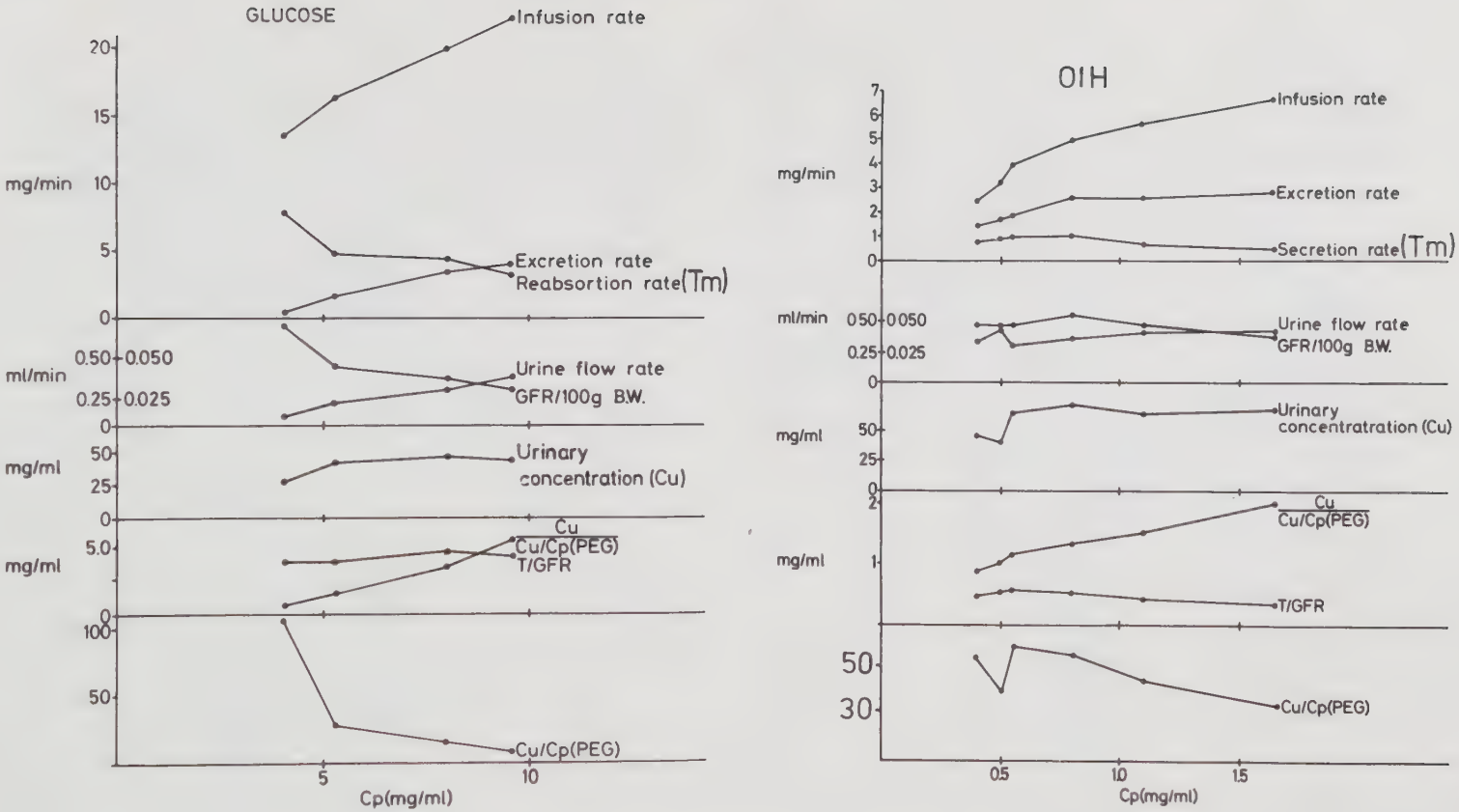

Fig. 1 The course of individual experiments for a. glucose and b. O-iodo-hippurate(OIH). The points represent the observed values for each clearance period in one kidney.

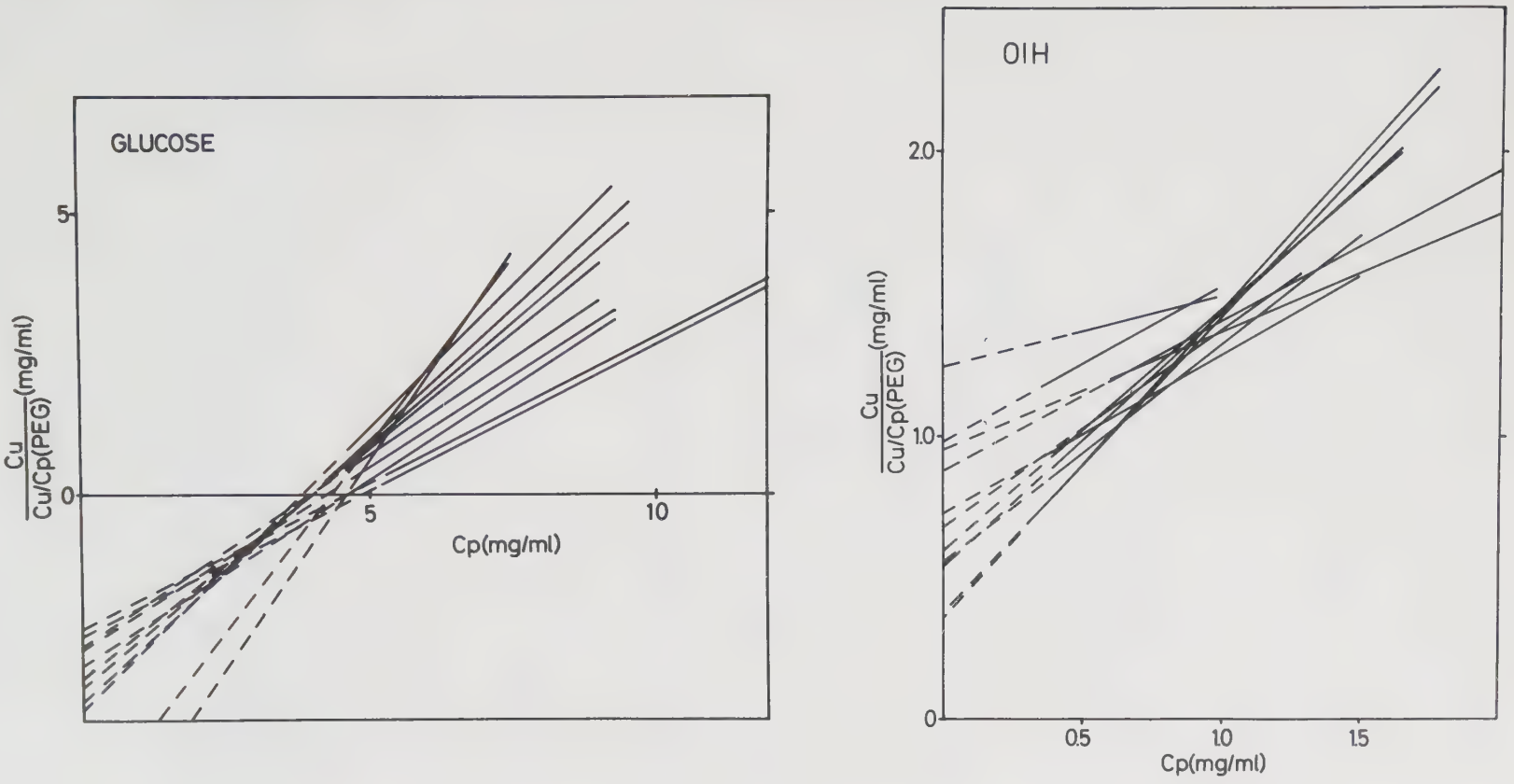

Fig. 2 The relation of $\frac{Cu}{Cu/Cp\ (PEG)}$ to plasma concentration (Cp) given as calculated regression lines for each kidney for a. glucose and b. O-iodo-hippurate (OIH). The lines are solid within the used Cp range.

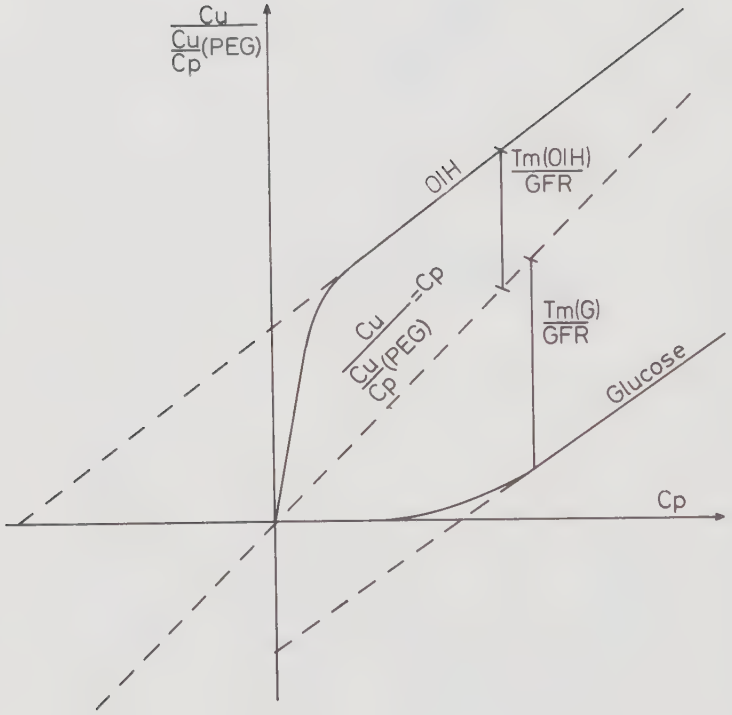

Fig. 3 The principal relationships between $\frac{Cu}{Cu/Cp\ (PEG)}$ and plasma concentration (Cp) for glucose and OIH. Tm/GFR equals the difference between $\frac{Cu}{Cu/Cp\ (PEG}$ and Cp (calculations eq. 4 and 5). A constant Tm/GFR gives a linear relationship with the slope 1.0. At the linear threshold Tm/GFR equals plasma concentration.

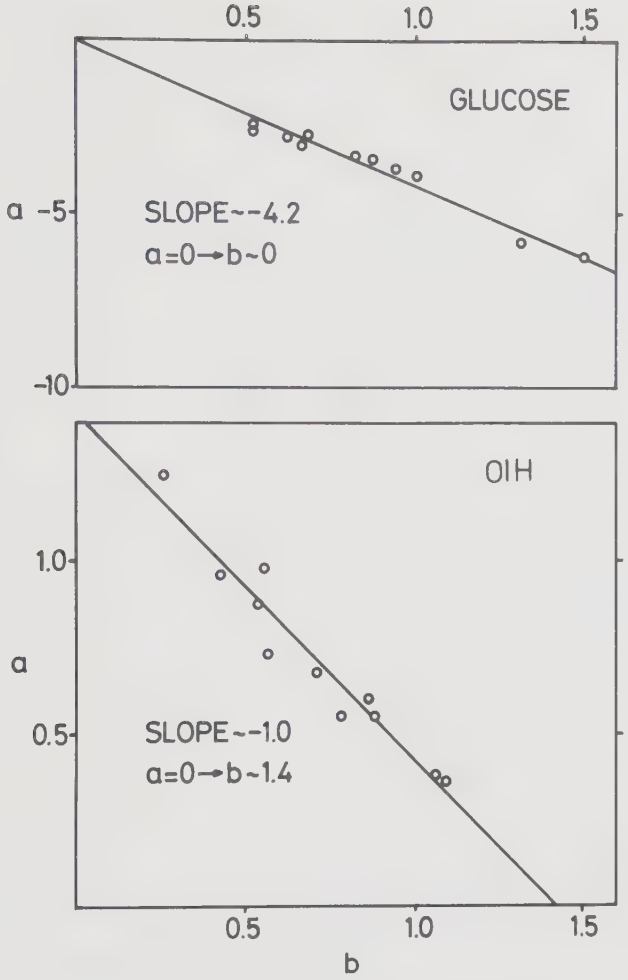

Fig. 4. Relation between values for a and b from the calculated regression lines with the formula $\frac{Cu}{Cu/Cp(PEG)} = a + b \cdot Cp$. The line of best fit is used.

the linear threshold for glucose, and at a Cp value near 1.0 mg/ml for Hippuran. To determine these intersection points the following calculations were performed: The point of intersection is denoted X′, Y′. The equation for the regression of $\frac{Cu}{Cu/Cp(PEG)}$ (= Y) on Cp (= X) is $Y = a + b \cdot X$. If all regression lines intersect at one point it follows that $Y' = a + b \cdot X'$ for all kidneys, but with individual a and b values for each kidney. The equation then can be transformed to $a = Y' - X' \cdot b$. The values for a and b from the calculated regression lines were plotted (Fig. 4). The plot showed that Y′ was about zero and X′about 4.2 for glucose.For Hippuran Y′ was about 1.4 and X′ about 1.0.

DISCUSSION

The purpose of this investigation was to study the effect of varying plasma concentration (Cp) on tubular transfer (Tm) of glucose and o-iodo-hippurate (OIH). For quantitative estimations of physiological events indirect measurements must be relied upon very often, e.g. in renal clearance investigations. The same primary values are frequently used for the calculation of several physiological variables. As pointed out by Wesson (1957) variables with common components are not independent and are therefore not suitable for statistical analyzis. This fact is very often disregarded. To avoid this difficulty when correlating Tm and GFR Engberg (1969) made an evaluation of errors in measurements, which showed that the variation of Tm/GFR could be explained by such experimental errors. As Tm has been reported to vary with GFR (Handley et al 1949), it seems reasonable to use Tm/GFR to estimate tubular transfer as a function of Cp. However, if Tm/GFR is correlated to Cp, the variables are not mathematically independent, as Cp is used to calculate Tm/GFR (see eq. 4 and 5). In the present study this problem is solved by relating the amount excreted per unit volume of GFR, $\frac{Cu}{Cu/Cp\ (PEG)}$ to Cp. The relation between Tm/GFR and Cp could then be established indirectly (eq. 4 and 5, Fig. 3).

In clearance studies GFR is used to calculate tubular transfer (eq. 2 and 3). A reliable GFR determination is important, particularly at high plasma concentrations of glucose and OIH, where the tubular transfer is small compared to the filtered amount. Inulin is generally accepted as the most reliable substance for measurement of GFR. However, there are indications, that in the rat inulin may not be freely filterable, and that different batches may give different values. In the rat polyethylene glycol, MW 800-1000 (PEG 1000) probably gives a more correct and a more reproducible value for GFR (2 + 3) and was therefore used in this study (Berglund 1965, Berglund et al 1969). If inulin gives the more correct value, clearance for PEG 1000 is 1.1 - 1.4 times higher than GFR. This will influence the absolute values for Tm/GFR and $\frac{Cu}{Cu/Cp\ (PEG)}$, and the slopes of the regression lines for $\frac{Cu}{Cu/Cp\ (PEG)}$ to Cp. The intersections of the regressio lines with the abscissa would, however, remain unaffected and there would still be a good linear correlation of $\frac{Cu}{Cu/Cp\ (PEG)}$ to Cp, with individual slopes that differ from 1.0 for most animals.

To detect a possible individual variation in tubular transfer of glucose and OIH, and to exclude the influence of such a variation in the investigation of the effect of varying plasma concentration, a titration curve was made for each animal. Thus $\frac{Cu}{Cu/Cp\ (PEG)}$ was correlated to Cp, and Cp was varied over a wide range in each animal. A linear correlation was found for each animal but with different slopes of

the individual regression lines (Table I, Fig. 2). As only one titration curve was made for each animal, it cannot be decided if this individual variation was due to differences between animals or between experiments. However, it could not be referred to any apparent variation in age, weight, preparation or other experimental conditions. A variation of extracellular fluid volume could be suggested. However, fluid substitution was carried out and body weight changes were small. With high plasma concentration of glucose a redistribution between extracellular and intracellular volume could have occurred.

In the experiments with isolated perfused rat kidneys by Bowman and Maack (1972) perfusate glucose concentration was negatively correlated to reabsorptive rate. Transport studies with perfused kidneys do however have several drawbacks (Bauman,Clarkson & Miles 1963, Nizet 1975). In micropuncture studies Baines (1971) found no variation between Tm (G)/GFR and plasma glucose in single nephrons. The published data do however not permit recalculation on individual rats.

The slopes of the regression lines differed from 1.0 for most animals. As a constant Tm/GFR should give a slope of 1.0 (see Fig 3 and Calculations, eq. 4 and 5), it is concluded that Tm/GFR varied with plasma concentration in most animals. As most of the slopes of the regression lines were below 1.0, Tm(OIH)/GFR decreased and Tm(G)/GFR increased with increasing Cp for most animals, indicating increased reabsorption for both substances. At very high plasma levels of OIH even net tubular reabsorption was observed. Similar results have been obtained earlier (Schachter & Freinkel 1951, Bryan & Maher 1973). An increase in fractional fluid reabsorption has to be considered as explanation . However the osmotic diuresis at high plasma concentrations of glucose and OIH would be expected to decrease the fractional fluid reabsorption (Baines 1971, Seely and Dirks 1969), which would act to decrease the transfer of glucose and OIH. A change in the active, saturable transport above threshold is a possibility that seems unlikely, because it would act in opposite directions for glucose and OIH. That diffusion across the tubular wall should be an important factor in net transfer of glucose and OIH, particularly at high plasma levels, has been suggested by Loeschke et al (1969) Bryan and Maher (1973) and Tune,Burg & Patlak (1969). A diffusion out from the tubules concords with increased reabsorption. At high plasma levels the filtered load would give a high tubular concentration of OIH, when fluid is reabsorbed. The same may also be true for glucose at high plasma concentrations, if glucose is not reabsorbed at the same rate as

water. According to eq. 6 in Calculations the endproximal tubular concentration (Ctf) of glucose and OIH can be estimated from the determined values of $\frac{Cu}{Cu/Cp\ (PEG)}$, and the experimentally observed values for fractional fluid reabsorption, reflected by Ctf/Cp (PEG). (Landwehr, Schnermann, Klose, Giebisch 1968, Lowitz, Stumpe, Ochwald 1968). From such a calculation it can be inferred that tubular concentration increased more rapidly than plasma concentration, and exceeded the plasma level even for glucose at high concentrations. This deduction supports the idea of a diffusion out of the tubules at high plasma levels. The calculation of Ctf requires the assumption that tubular transfer of glucose and OIH ceases at the endproximal tubule segment. For the time being this assumption seems relevant as the transfer in the distal parts of the nephron is found to be small (Frohnert, Höhmann, Zwiebel, Baumann 1970).

It has been reported from in vitro experiments that OIH is not freely filterable due to plasma protein binding (Bryan & Maher 1973). However, these ultrafiltration experiments were performed at lower temperatures than in vivo clearances and since it is known that increasing temperature increases the ultrafilterable fraction of PAH (Rahill 1970) the filterability of OIH in in vivo experiments can not be predicted. Furthermore recent in vivo experiments have shown similar clearances for PAH and OIH when urine samples were collected from the ureters (Mushmov, Brailska, Budevski, Salambashev, Dimitrova 1973). PAH probably has some protein binding although the concentration of PAH and OIH in the space of Bowman has not yet been determined. If OIH would not be freely filtrable the slope of the regression lines of $\frac{Cu\ (OIH)}{Cu/Cp\ (PEG)}$ to Cp (OIH) should be increased and the tendency towards increased reabsorption should be diminished or even eliminated. The individual variation and the intersection point should, however, remain unchanged. For OIH "self depression" of secretion at high plasma levels is also reported (Schachter & Freinkel (1951)). This phenomenon could be explained by back-diffusion of OIH to plasma. At high plasma levels of glucose a reduced GFR is seen in this study as is also described earlier (Dempster, Eggleton & Shuster 1956). As Tm/GFR varies with Cp for both glucose and OIH, but GFR only for glucose, the variation of Tm/GFR with respect to GFR more probably reflects a dependance of both GFR and Tm(G)/GFR on Cp. The fall in GFR at high plasma concentrations of glucose might possibly be explained as a consequence of increased serum osmolality (Fredriksson 1973).

Rather welldefined points of intersection of the individual regression lines $\frac{Cu}{Cu/Cp\ (PEG)}$ to Cp are obtained in the present study for both the glucose and the OIH groups, respectively (see results). Further investigations to confirm

these results would be very interesting as these might be the points where the specific active transport mechanism is best studied in clearance investigations. A reasonable speculation would be that the active transfer mechanism best reflects the cell function. At higher plasma concentrations other factors as diffusion and bulk flow may play a greater role for net transfer of these test substances. The intersection point for glucose was at the linear threshold, i.e. where the regression lines of $\frac{Cu(G)}{Cu/Cp\ (PEG)}$ on Cp (G) intersect with the abscissa. For OIH it was at a plasma concentration of 1.0 mg/ml. It is possible that measurements of tubular transfer at these points are preferable to the use of Tm and Tm/GFR in estimations of proximal tubular epithelial lesions in renal disease. (Fredriksson, Engberg, Persson, Hamberg, 1975).

The tubular transfer of glucose and OIH is a complex process. It includes active transport mechanisms and it could also be influenced by bulk flow and diffusion. The dependence on GFR, fractional fluid reabsorption and plasma concentration has to be considered when using transfer maxima of glucose and OIH to estimate proximal tubular function with clearance methods.

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TUBULAR TRANSFER OF GLUCOSE AND O-IODO-HIPPURATE IN EXPERIMENTAL RENAL DISEASE

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ABSTRACT

The tubular transfer (Tm) of glucose and o-iodo-hippurate (OIH) was studied in clearance experiments on rats with induced pyelonephritis or ischemic renal injury. The plasma concentration of glucose and OIH was varied over a wide range in the individual animals. Glomerular filtration rate (GFR) was estimated as clearance for polyethylene glycol, MW 800-1000 (PEG 1000). As in a previous study in normal rats, the amount of glucose or OIH excreted per unit volume of glomerular filtrate was correlated to plasma concentration for each rat. A linear correlation was seen in individual animals. The Tm and Tm/GFR varied with plasma concentration with a decreased fractional excretion at high plasma levels for both glucose and OIH. In the study on normal rats it was suggested, that a suitable method to estimate tubular transfer of glucose and OIH might be at common "intersection points" of the individual regression lines, which for glucose was the linear threshold, and for OIH the amount excreted per unit volume of glomerular filtrate at a plasma concentration of 1.0 mg/ml. The intersection points for normal animals were employed in this study, and for the diseased animals the values were usually lower than for normals. In unilateral renal disease the intersection point values were usually decreased both for the diseased and the intact kidney, but for OIH they were lower in the diseased kidneys. It is concluded that the tubular transfer of glucose and OIH, when recorded in this manner, is decreased both by tubular epithelial lesions and by a functional adaptation in renal disease.

Key words: Kidney function; proximal tubules; experimental renal disease; glucose and o-iodo-hippurate transport; kidney function tests.

In many of the common renal diseases an impaired function of the proximal renal tubules is to be expected from the pathogenesis and morphology (Hoang Tuan, Hageman, Briedigkeit & Dutz, 1972; de Wardener, 1973). As the active tubular transfer of glucose and para-amino-hippuric acid (PAH) mainly occurs in the proximal tubules (Pitts, 1968), the maximal transfer capacity for these substances has been used as a test for proximal tubule function. However, the relation between tubular transfer, plasma concentration and glomerular filtration rate (GFR) in normal kidneys has been the object of much discussion (cf. Mudge, Berndt & Valtin, 1973; Weiner, 1973). Numerous attempts have also been made to characterize proximal tubule function in diseased kidneys utilizing substances like glucose and PAH. In unilateral renal disease glucose and PAH excretion have been reported to vary in proportion to GFR. However, in uremia this relation seems to be different probably due to functional adaptation (Bricker, Klahr, Lubowitz & Rieselbach, 1965; Bricker, 1969; Bricker, Klahr, Lubowitz & Slatopolsky, 1971; Seldin, Carter & Rector, 1971).

In a previous study (Fredriksson, Engberg, Persson & Ulfendahl, 1975) using normal rats we have found that both Tm and Tm/GFR varied with plasma concentration of glucose and o-iodo-hippurate (OIH) even in individual rats. However, it was found that if the excretion of glucose and OIH was expressed as the amount excreted in urine per unit volume of glomerular filtrate and measured as a function of plasma concentration, there was a linear correlation for individual rats and there was also a common intersection point for all the individual regression lines for glucose and OIH respectively. Thus these intersection points seemed to be independent of individual variations and plasma concentration of glucose and OIH, and were therefore regarded as suitable points to measure proximal tubular function. Therefore we performed similar experiments in rats with different types of renal disease. In the diseased kidneys it was found that Tm and Tm/GFR did vary with plasma concentration, as for normal animals, and the relation between the amount of glucose or OIH excreted per ml glomerular filtrate as a function of plasma concentration gave a linear relation for individual rats. Furthermore, in diseased kidneys there was a fall in the intersection point values, indicating decreased tubular transfer. However, the intersection point values of the intact kidneys in unilateral renal disease also decreased compared to normal animals. Thus both tubular epithelial lesions and func-

tional adaptation in renal disease seemed to influence the values recorded.

MATERIAL AND METHODS

Animals

Thirty-six male albino rats of the Sprague-Dawley strain (Anticimex, Stockholm, Sweden) were used. Their weight at the induction of kidney damage was approximately 250 g. Since the animals were studied at different time intervals from the induction of kidney damage, their individual weights at the time of clearance investigation varied between 240 and 500 g. The rats were fed on standard diet (Ewos, Södertälje, Sweden), and had free access to food and water until the start of the experiments.

Experimental groups

Pyelonephritis was induced in the left kidney in two ways. The kidney was exposed by an abdominal incision during ether anaesthesia. In one group (Py 1, n = 12) a method modified from that described by Shankel, Robson & Bricker (1967) was used. The kidney was punctured one hundred times with a needle that had been repeatedly dipped into a culture of enterococci (10^{10} bact/ml). In a second group (Py 2, n = 4) 0.15 ml of the enterococcal culture was injected into the renal pelvis at the beginning of 30 minutes of ureteral occlusion. In the groups Py 1 and Py 2 the right kidney was left intact. In a third group (Py S, n = 4) the left kidney was treated in the same way as in Py 1, but the right kidney was removed simultaneously. The functional studies were performed 2 to 4 months after induction of pyelonephritis.

Bacterial cultures were made from urine samples, obtained at the start of the clearance experiments, by needle aspiration from the exposed bladder during anaesthesia. Quantitative cultures were made from kidney sections, cut out at sacrifice with a disinfected but not sterile knife. The samples were stored at +4°C until serial dilutions in phosphate buffered saline and cultures on blood agar plates were performed.

Cultures showed more than 10^5 bact/ml of urine in about 60 % of the pyelonephritic animals. Renal tissue showed more than 10^4 bact/g for both right and left kidneys when urine cultures were positive. No other bacteria than enterococci were found.

Table I. Characteristics of the experimental groups

Group	Body weight g	Kidney weight g	GFR/100 g body weight ml/min
Normal[a] (12)	419 ± 13		
right		1.64 ± 0.05	0.42 ± 0.01
left		1.70 ± 0.06	0.44 ± 0.02
Pyelonephritis			
Py 1 (12)	432 ± 10		
right kidney		2.13 ± 0.09	0.37 ± 0.03
left kidney		1.59 ± 0.07	0.22 ± 0.02
Py 2 (4)	430 ± 16		
right kidney		1.92 ± 0.13	0.40 ± 0.02
left kidney		1.74 ± 0.11	0.34 ± 0.02
Py S (4)	454 ± 9		
left kidney		3.28 ± 0.28	0.39 ± 0.07
Ischemic renal injury			
40 min, I 40 (8)	299 ± 11		
right kidney		1.78 ± 0.06	0.48 ± 0.08
left kidney		1.54 ± 0.11	0.16 ± 0.05
60 min, I 60 (8)	296 ± 4		
right kidney		1.94 ± 0.11	0.60 ± 0.12
left kidney		1.24 ± 0.20	0.07 ± 0.01

[a] Reference no. 4

Values are given as mean ± SEM

The numbers in parentheses denote the number of animals

When the functional studies were completed, the animals were killed by intraarterial air injections. The kidneys were excised, freed from the loose, adipose capsule, immediately weighed, longitudinally sectioned and fixed in 10 % formaldehyde solution. Deparaffinized, haematoxylin-eosin stained sections were studied by light microscopy.

The left kidneys in Py 1 were contracted with a scarred surface. Their weight

was reduced compared to the right kidneys (Table I), which were grossly normal. Light microscopy showed moderate chronic-subchronic interstitial nephritis in the left kidneys, and minimal changes were found in some of the right kidneys. In Py 2 the left kidneys showed only a few superficial scars, and their weight was only slightly reduced. Light microscopical findings were essentially as in Py 1. In Py S the kidneys were hypertrophied, irregularly shaped and had a greyish discolouration. Their weight was greatly increased compared to the kidneys in the other groups. Light microscopy showed somewhat more marked inflammatory changes than in Py 1 and Py 2, and also more tubular dilatation.

Ischemic renal injury was induced by temporary occlusion of the left renal artery with a 0000 silk thread during anaesthesia with intraperitoneally injected Nembutal® (Abbot, Queensborough, Kent, England). Body temperature was held constant at 37 ± 0.5°C with a servocontrolled heating pad. The ischemia time was 40 minutes in one group (I 40, n = 8) and 60 minutes in one group (I 60, n = 8). The right kidneys were left intact. Functional studies were performed 11 to 15 days later. Morphological studies were made as for the pyelonephritic animals.

The left kidneys, which underwent temporary ischemia, were contracted, with reduced weight compared to that of the right kidneys (Table I). On light microscopy the tubules were dilated throughout with flattened epithelial cells and some casts. The changes were most advanced in the inner medullary zone and cortico-medullary junction. There were necrotic areas surrounded by mononuclear infiltration. The glomeruli appeared normal with the exception of those in the necrotic areas. In the right kidneys only moderate hypertrophy was detected.

All animals were in a good general condition and gained weight at a normal rate. Blood pressure during Inactin-anaesthesia was within the normal range. Values of GFR are given in Table I.

Clearance experiments

The experiments were performed during Inactin®-anaesthesia, by the same procedure as applied to studies on normal rats (Fredriksson et al, 1975). GFR was estimated as clearance of polyethylene glycol, molecular weight 800-1000 (PEG 1000).

The following infusions were given:

Glucose experiments:

Glucose 50 % in water	2.9 - 5.0 ml/h
^{3}H or ^{14}C-PEG 1000[a], 0.25 µCi/mg, 4 mg/ml, in saline	0.6 - 1.0 ml/h

O -iodo-hippurate experiments:

Ortho-I-hippurate sodium[b], 150 mg/ml, ortho-I-hippurate sodium labelled with ^{125}I or ^{131}I[c], 5 µCi/ml, in saline	1.0 - 2.7 ml/
PEG 1000[d], 200 mg/ml, in saline	1.0 ml/h

Analytical methods

The same methods were used here as in studies on normal rats (Fredriksson et al, 1975). ^{3}H was treated like ^{14}C.

Calculations

The following abbreviations are used:

GFR = glomerular filtration rate, ml/min
$\dot{V}$ = urine flow rate, ml/min
Tm = tubular transfer above threshold, mg/min
Cp = concentration in plasma, mg/ml
Cu = concentration in urine, mg/ml
(PEG) = PEG 1000, polyethylene glycol, molecular weight 800-1000
(G) = glucose
(OIH) = ortho-I-hippurate sodium

The following conventional equations were used:

$$GFR = \dot{V} \cdot Cu/Cp\ (PEG) \qquad (1)$$

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a NEN, Frankfurt, West Germany
b Hippuran, Mallinckrodt, New York, USA
c AB Atomenergi, Studsvik, Sweden
d Kebo, Stockholm, Sweden

Glucose excretion rate,

$$Cu\,(G) \cdot \dot{V} = GFR \cdot Cp\,(G) - Tm\,(G) \qquad (2)$$

OIH excretion rate,

$$Cu\,(OIH) \cdot \dot{V} = GFR \cdot Cp\,(OIH) + Tm\,(OIH) \qquad (3)$$

Rearranging of equations (2) and (3) gives:

$$Tm\,(G)/GFR = Cp\,(G) - \frac{Cu\,(G)}{Cu/Cp\,(PEG)} \qquad (4)$$

$$Tm\,(OIH)/GFR = \frac{Cu\,(OIH)}{Cu/Cp\,(PEG)} - Cp\,(OIH) \qquad (5)$$

$\frac{Cu}{Cu/Cp\,(PEG)}$ for glucose and OIH were correlated to Cp.

Regression lines were calculated for each kidney according to the formula

$$\frac{Cu}{Cu/Cp\,(PEG)} = a + b \cdot Cp \qquad (6)$$

Statistical analyzes were made according to Snedecor & Cochran (1967). Only observations well above the threshold were included. Of a total number of 314 observations 5 were excluded, as they differed greatly from the majority.

RESULTS

The amount of glucose or OIH excreted per unit volume of glomerular filtrate, $\frac{Cu\,(G)}{Cu/Cp\,(PEG)}$ and $\frac{Cu\,(OIH)}{Cu/Cp\,(PEG)}$, respectively, was correlated to Cp for individual rats. A linear correlation was seen in most animals ($p < 0.001$). The regression lines for the different groups are illustrated in Fig. 1. For the great majority of animals the slopes were below 1.0 ($p < 0.001$). If Tm/GFR was constant, the slopes would be 1.0, as shown in a previous study on normal rats (Fredriksson et al, 1975). For all groups except Py 2 there was no significant difference between the slopes of the regression lines of individual animals and no common intersection points. For glucose in the group Py 2 (Fig. 1) the picture was similar to that found for normal rats (Fredriksson et al, 1975); the slopes differed between individual animals

Table II. Mean differences between $\frac{Cu}{Cu/Cp\ (PEG)}$ for left and right kidney for all clearance periods

Group	Glucose n	Glucose mean (p = 0.95)	O-iodo-hippurate n	O-iodo-hippurate mean (p = 0.95)
Normal[a]	5	0.08 ± 0.40	6	-0.06 ± 0.08
Pyelonephritis				
Py 1	8	-0.32 ± 0.36	4	-0.042 ± 0.025[x]
Py 2	4	-0.43 ± 0.69		
Ischemic renal injury				
I 40	4	0.94 ± 1.26	4	-0.159 ± 0.095[x]
I 60	4	2.09 ± 3.09	4	-0.136 ± 0.219

n Number of rats
a Reference no. 4
x Significant difference (p = 0.95, confidence limits)

but not between right and left kidneys in the same animal; there was a rather welldefined common intersection point for the regression lines near the linear threshold.

To investigate the functional impairment in the diseased kidneys, compared to the intact kidneys in unilateral renal disease, the mean differences between $\frac{Cu\ (G)}{Cu/Cp\ (PEG)}$ or $\frac{Cu\ (OIH)}{Cu/Cp\ (PEG)}$ for left and right kidneys for all clearance periods were calculated (Table II). This difference is the same as the difference between Tm/GFR (see eq. 4 and 5). For each individual rat the difference did not change sign with varying plasma concentration. Significant differences (p = 0.95, confidence limits) were found for OIH in Py 1 and I 40. The differences were of the same order as between the individual animals.

From the study in normal rats it was suggested, that the intersection points of the regression lines of $\frac{Cu}{Cu/Cp\ (PEG)}$ as a function of plasma concentration, would be suitable points to measure tubular transfer of glucose and OIH. The intersection point for glucose in normal rats (Fredriksson et al, 1975) was at the intersection of the regression lines with the abscissa,

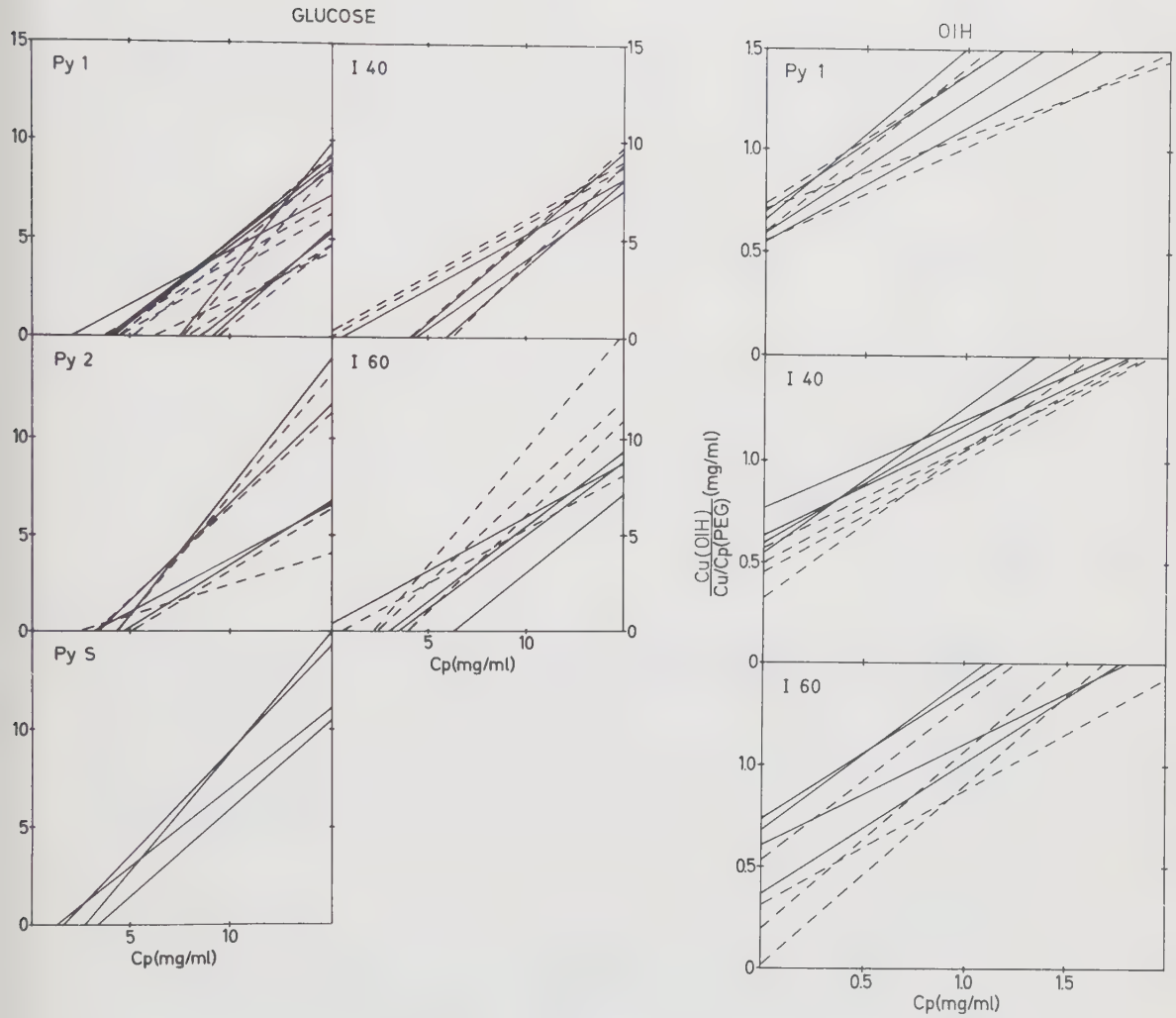

Fig. 1. Regression lines of the relation of $\frac{Cu}{Cu/Cp\ (PEG)}$ to plasma concentration for glucose and o-iodo-hippurate (OIH) for the individual animals in the different experimental groups. For animals with both kidneys remaining represents right kidney, and --- left kidney.

Table III. Comparison between the experimental groups at the intersection points

Group	Glucose $\left\lvert\frac{a}{b}\right\rvert$ n	Glucose $\left\lvert\frac{a}{b}\right\rvert$ mean (p = 0.95)[b]	O-iodo-hippurate (a+b) n	O-iodo-hippurate (a+b) mean (p = 0.95)[b]
Normal[a]				
right	6	4.17 ± 0.34	6	1.41 ± 0.08
left	5	4.32 ± 0.45	6	1.44 ± 0.10
Pyelonephritis				
Py 1				
right kidney	8	5.80 ± 2.04	4	1.28 ± 0.26
left kidney	8	5.93 ± 1.66	4	1.21 ± 0.29
Py 2				
right kidney	4	3.68 ± 1.17		
left kidney	4	3.46 ± 2.09		
Py S				
left kidney	4	2.23 ± 1.68		
Ischemic renal injury				
40 min, I 40				
right kidney	4	2.97 ± 2.94	4	1.19 ± 0.11
left kidney	4	2.29 ± 5.14	4	1.04 ± 0.08
60 min, I 60				
right kidney	4	2.82 ± 4.34	4	1.24 ± 0.31
left kidney	4	2.03 ± 2.12	4	1.08 ± 0.29

n Number of animals
a Reference no. 4
b Confidence limits

For glucose $\left\lvert\frac{a}{b}\right\rvert$ = Cp when $\frac{Cu}{Cu/Cp\ (PEG)}$ = 0 mg/ml

For o-iodo-hippurate (a+b) = $\frac{Cu}{Cu/Cp\ (PEG)}$ when Cp = 1.0 mg/ml

i.e. the linear threshold, equal to a/b in eq. 6. For OIH it was when plasma concentration was 1.0 mg/ml. The results are seen in Table III and Fig. 2; the values from normal rats are included for comparison. The scatter of the values was much larger in all disease groups than for normals, both

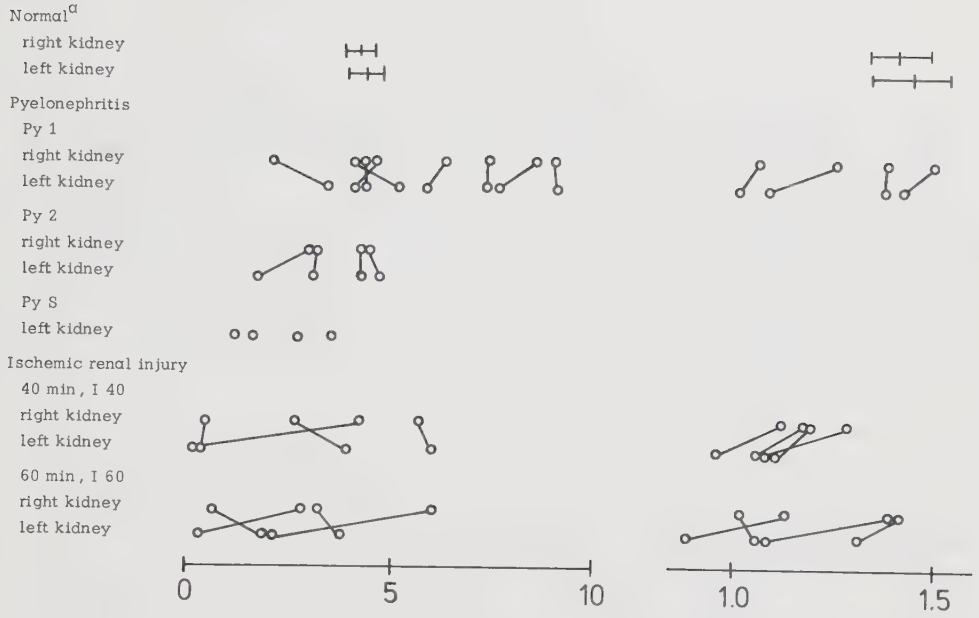

Fig. 2. Values at the "intersection points" for the individual animals in the different experimental groups. For glucose the intersections of the regression lines with the abscissa are used for calculations, and for o-iodohippurate the values of $\frac{Cu\ (OIH)}{Cu/Cp\ (PEG)}$ at a plasma concentration of 1.0 mg/ml. For animals with unilateral renal disease, the points for the right and left kidney are connected with a line. For normal animals(ref. no. 4) are shown the mean $\pm$ 0.95 confidence limits.

for diseased and intact kidneys in unilateral disease. The mean intersection point values were lower than for normal animals for both glucose and OIH, except for glucose in group Py 1. These differences were significant for glucose when Py S was compared to normals or Py 1, and for OIH when I 40 left or right kidneys were compared to normal kidneys ($p = 0.95$). Lower values mean a decreased reabsorption of glucose and a decreased excretion of OIH, i.e. a decreased tubular transfer. It has to be noted, that both kidneys in the same animal commonly gave values that differed in the same direction from normals. However, with OIH all but one of the left diseased

kidneys gave lower values than the corresponding intact kidneys in unilateral renal disease (Fig. 2).

DISCUSSION

The histological alterations found in the pyelonephritic kidneys in this study were similar to those observed in one of the most common renal pathological entities, namely chronic interstitial nephritis. Some degree of proximal tubular dysfunction could be expected, as there are structural changes in the tubules (Schirmeister, 1966), as the bacteria probably invade the interstitium through the tubules (Hoang Tuan et al, 1972), and as also tubular dysfunction may be correlated to interstitial changes (Schainuck, Striker & Cutler, 1968).

In the ischemically injured kidneys there were marked generalized changes in the tubules, without signs of glomerular damage. This model therefore should be suitable to study the effect of tubular damage on renal function. It is probable that the reduction of GFR could only partly be explained by necroses. As glomeruli outside the necrotic areas were structurally normal a feedback-induced reduction of GFR due to the tubular lesion could be suggested (Mason, Takabatake, Olbricht & Thurau, 1974). To obtain reproducible renal damage, it is important to control the body temperature during temporary occlusion of the renal artery. In pilot studies without control of body temperature the extent of tubular injury varied considerably both with barbiturate and ether anaesthesia.

In this investigation a linear correlation between the excreted amount per unit volume of glomerular filtrate, $\frac{Cu}{Cu/Cp\ (PEG)}$, and plasma concentration of glucose and o-iodo-hippurate (OIH) was found, as for normal kidneys (Fredriksson et al, 1975). As most of the slopes were below 1.0, Tm (G)/GFR rises and Tm (OIH)/GFR falls with increasing plasma concentration as for normal rats. This means that there is a decreased fractional excretion of both glucose and OIH at high plasma levels, probably due to diffusion from tubular lumen to blood at high plasma concentrations (Fredriksson et al, 1975).

When the tubular transfer of glucose and OIH is compared between animals or groups of animals, the variation with GFR and plasma concentration has

to be considered. As Tm varies with GFR (Mudge et al, 1973; Weiner, 1973) and Tm/GFR with plasma concentration (Fredriksson et al, 1975), they seem inappropriate to use. Tm/GFR usually fails to detect tubular dysfunction in unilateral renal disease, as shown earlier (Bricker et al, 1965; Bricker et al, 1971) and in the present investigation (Table II). One criterion for a suitable measure should be a small interindividual variation in normal animals. Therefore a comparison at the so called "intersection points" found for normal rats is made, although no corresponding points are found in most of the diseased groups. No more suitable point is indicated by the regression lines for the diseased groups (Fig. 1). The lack of common intersection points in the disease groups may be explained by a variability in the degree of damage. The intersection points also could be suitable to detect tubular epithelial dysfunction, as they may be the points where active tubular transfer is best reflected, and where other transport mechanisms, such as diffusion and volume flow are of least importance, as discussed in the study on normal rats (Fredriksson et al, 1975).

In the diseased animals in the present study the values for the intersection points (Table III, Fig. 2) were lower than for normal animals, although significantly only for three groups. These lower values would indicate a decreased tubular transfer of glucose and OIH. In individual animals a difference between diseased and intact kidneys is seen for OIH, particularly in ischemic renal injury, with marked tubular damage, but there are equally large or even larger differences between diseased and normal animals and between animals within the disease groups. For glucose there are consistent differences only between animals. This indicates that the intersection point values are not only influenced by the tubular epithelial lesion, but also by some type of functional adaptation which occurs in renal disease. This conclusion is further supported by a difference in the intersection point values between the diseased kidneys in Py 1, with one diseased and one intact kidney, and Py S with the same induced renal injury, but with the intact kidney removed. The large scatter of the values in the diseased groups compared to normal animals, may also be taken as an indication of abnormal function.

These results are in agreement with earlier studies of proximal tubular functions in unilateral renal disease with and without a contralateral intact kidney (Bricker et al, 1965; Bricker et al, 1971), and with the intact nephron hypothesis as stated by Bricker (1969). However, more evidence of the

tubular epithelial, lesion seems to be given by the method employed here. It would be of great interest to have a substance which more specifically reflects tubular epithelial dysfunction, but not functional adaptation in renal disease. A low molecular weight protein might prove to be valuable (Peterson, Evrin & Berggård, 1969). The influence of the functional adaptation in renal disease upon the intersection point values could be interpreted as if the tubular dysfunction was counterbalanced by another factor. A method that unmasks this balance could make it possible to detect the tubular lesion.

CONCLUSIONS

To measure the proximal tubular transfer, as the linear threshold for glucose, and as the amount excreted per volume unit of glomerular filtrate at a plasma concentration of 1.0 mg/ml for o-iodo-hippurate seems to be more valid than to measure Tm or Tm/GFR. The transfer so measured seemed to be decreased both by tubular epithelial lesions and by a functional adaptation in renal disease.

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EFFECTS OF RENAL DYSFUNCTION ON β_2-MICROGLOBULIN METABOLISM

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ABSTRACT

The metabolism of human β_2-microglobulin has been investigated in normal rats and in rats with kidney dysfunction. Severe damage of the renal tubular function could be induced in the rats by injections of low doses of the nephrotoxic agent sodium maleate. Higher doses of sodium maleate caused reduction of the glomerular filtration rate (GFR) in addition to tubular damage.

The fractional metabolic rate (Fmr) for β_2-microglobulin in normal rats and in ureter-ligated animals was approximately 6-fold higher than in bilaterally nephrectomized rats. The importance of the kidney in the elimination of β_2-microglobulin from plasma was further assessed by the difference between the β_2-microglobulin concentrations in arterial and renal vein blood.

In rats with tubular impairment Fmr of β_2-microglobulin was normal, but a shift had occurred from catabolism to proteinuria. In tubular damage combined with a reduced GFR, the Fmr of β_2-microglobulin was decreased, as in ureter-ligated rats with impaired tubular function.

All data obtained were compatible with the view that small proteins like β_2-microglobulin are rapidly filtered across the glomerular barrier. Most of the filtered protein is subsequently taken up and degraded by the tubular cells. In conditions of reduced GFR the catabolism is diminished. Damage of tubules will according to this view also result in diminished catabolism of small proteins in spite of a normal fractional metabolic rate.

Key words: β_2-microglobulin; renal protein metabolism; proteinuria; renal tubular dysfunction; experimental.

INTRODUCTION

It is generally agreed that plasma proteins are handled by the kidney by two consecutive processes: glomerular filtration and tubular reabsorption (cf. Schultze & Heremans, 1966). The classical studies by Wallenius (Wallenius, 1954; Arturson & Wallenius, 1964) and later investigations by Hardwicke & Hulme (Hardwicke & Hulme, 1967; Hardwicke, Hulme, Jones & Ricketts, 1968; Hulme & Hardwicke, 1968) have shown that size is an important factor in the urinary excretion of neutral macromolecules. However, a number of investigations have clearly shown that the renal handling of plasma proteins is governed by factors of a greater complexity and the process of their glomerular filtration is far from understood (cf. Schultze & Heremans, 1966; Berggård, 1970). The tubular reabsorption of filtered plasma proteins has been demonstrated by a variety of techniques (cf. Schultze & Heremans, 1966). Sparse knowledge is, however, at present available concerning the possible importance of factors like size and charge for the tubular uptake of proteins.

The glomerular filtration effectively eliminates low molecular weight substances from plasma whereas the excretion of large molecules is greatly restricted. The abundance of low molecular weight proteins in urine of patients with tubular proteinuria due to impaired tubular reabsorption of filtered plasma proteins has suggested that small plasma proteins are easily filtered across the glomerular barrier (Creeth, Keckwick, Flynn, Harris & Robson, 1962; Flynn & Platt, 1968; Piscator, 1966; Harrison, Lunt, Scott & Blainey, 1968; Peterson, Evrin & Berggård, 1969). From such observations it may be inferred that the kidney is of importance in the metabolism of low molecular weight plasma proteins. A number of investigations provide support for this view (Solomon, Waldmann, Fahey & McFarlane, 1964; Wochner, Strober & Waldmann, 1967; Hansen, Karle & Andersen, 1971; Vahlquist, Peterson & Wibell, 1973).

The urinary excretion of β_2-microglobulin in patients with well defined tubular or glomerular lesions is previously described (Peterson et al, 1969). The data obtained in that study indicated that β_2-microglobulin may be a suitable protein for evaluation of some features of the renal processing of small plasma proteins. Among the attractive properties of β_2-microglobulin is its appearance as a monomer in serum and urine, its low molecular weight (11 800), and its charge homogeneity (Berggård & Bearn, 1968). The results

in this communication show that the main catabolic site for β_2-microglobulin is the kidney. Furthermore, the data indicate that both tubular and glomerular dysfunction may affect the metabolism of this low molecular weight protein.

MATERIAL AND METHODS

Animals

In all studies male Sprague-Dawley rats weighing 250 to 325 g were used.

Protein labelling procedure

Human β_2-microglobulin, purified essentially as described by Berggård & Bearn (1968), was labelled with ^{125}I (Amersham, England) by the iodine monochloride method of McFarlane (1958). No preoxidation was employed. The preparation of the final product used for the turnover studies contained less than 1.5 % of non-precipitable radioactivity. Purity, unaltered electrophoretic mobility, and retained immunoreactivity of the iodine labelled β_2-microglobulin was assessed by crossed immunoelectrophoresis according to Ganrot (1972). By this criterion the labelled protein was indistinguishable from its native counterpart.

Experimental procedure

The animals were anaesthetized by intraperitoneal injection of long-acting barbiturate (Inactin®, Promonta, Hamburg), 120 mg/kg body weight. They were tracheostomized and the body temperature was held at 37 ± 0.5°C by a thermostat -controlled heating pad. Catheters were inserted into a femoral artery for sample injection and blood collection. Urine was collected through catheters in the distal ureteral ends. The injected sample of β_2-microglobulin ranged from 5 to 250 µg, dissolved in 0.5 ml of 0.02 M Tris-HCl buffer, pH 7.4, containing 0.15 M NaCl. Blood samples (0.1 ml) were withdrawn at intervals indicated in the figures. Isotonic saline was injected to compensate for fluid losses. Renal vein samples were taken through an indwelling catheter via the adrenal vein.

Bilateral nephrectomy was performed through a midline abdominal incision, that was closed before the turnover studies were started. Ureteral ligation was performed as a double ligature at the distal ureteral ends. Turnover studies were started in half an hour after ligation or nephrectomy.

Sodium maleate (Sigma Chemical Co.) adjusted to pH 7.4 with NaOH was administered intravenously by slow injection since the solution was hyperosmolar (1.5 M). One hour after the appropriate amount of sodium maleate had been administered, the experiments were started by injection of β_2-microglobulin or polyvinylpyrrolidone (PVP). After the completion of the metabolic studies the kidneys were fixed in formaldehyde solution. Deparaffinised, haematoxylin-eosin stained sections were studied by light microscopy.

The protein-bound radioactivity in plasma samples was recovered by precipitating the proteins with 10 % icecold trichloroacetic acid. ^{125}I-labelled β_2-microglobulin excreted into the urine was obtained in the same way. To avoid losses of precipitable protein, 0.1 ml of 10 % serum albumin was added to the urine samples prior to precipitation. Radioactivity of the precipitates was measured in a Tricarb 3003 gamma spectrometer (Packard Instrument Co., Chicago, Ill.) and counting was performed until a statistical error of less than 2 % was obtained.

Procedure for calculation

The logarithm of the protein-bound radioactivity was plotted against time. The zero time value was obtained by dividing the administered dose of radioactivity with the plasma volume, assumed to be 4 % of the body weight. Kinetic data were calculated according to the procedure devised by Nosslin (1966). The plasma disappearance curve for the labelled protein could thus be resolved into a series of exponential functions each defined by its slope b_i and its intercept of the ordinate c_i. The area under the disappearance curve (A) was calculated by integration ($A = \sum_{1-n} c_i/b_i$). The fractional metabolic rate (Fmr) is obtained as 1/A. The biological half life (BHL) is given by ($0.693 \cdot B/A$), where B equals ($\sum_{1-n} c_i/b_i^2$). The percentage of the total β_2-microglobulin pool present in the intravascular compartment (% I.V.) is equal to $100A^2/B$.

The proteinuric rate is here defined as the fraction of the intravenous pool which appears in the urine as intact protein per unit time. The percentage of overall metabolism resulting from proteinuria was calculated from the number of counts excreted as protein in 2.5 hours (multiplied by 100) divided by the total number of counts lost during the same time period to catabolism and proteinuria. The value so obtained was multiplied by the Fmr to yield the Fpr (fractional proteinuric rate) (Mogielnicki, Waldmann &

Strober, 1971).

Measurement of renal clearance of polyvinylpyrrolidone (PVP)

Measurement of renal clearance of PVP was performed in normal and sodium maleate treated rats essentially as described by Hardwicke et al. (1968). ^{125}I-labelled PVP (Amersham, England) was administered to rats through a femoral artery. Urine was collected for one hour. At the beginning and end of this period blood samples were drawn. The serum and urine samples to which 0.1 ml of 10 % serum albumin had been added were fractionated by gel chromatography on a column (70 x 1 cm) of Sephadex G-200 (Pharmacia Fine Chemicals, Uppsala, Sweden) equilibrated with 0.15 M NaCl. The molecular radii of PVP in serum and urine were calculated according to Laurent and Killander (1964) using albumin as an internal standard in each run. The clearance, expressed as the ratio of urinary PVP to plasma PVP is given in percentage of the average glomerular filtration rate (GFR) measured as endogenous creatinine clearance in a group of normal rats. The plasma PVP values were obtained as the logarithmic mean of the two samples collected at the beginning and the end of the urine collection period.

Induction of tubular and glomerular dysfunction

It is well known that sodium maleate is a nephrotoxic agent (Berliner, Kennedy & Hilton, 1950; Harrison, H.E. & Harrison, H.C., 1954; Rosenberg & Segal, 1964) affecting tubular as well as glomerular function (Mogielnicki et al, 1971). It has, however, also been shown that there exists a dose-response relationship between the amount of sodium maleate injected and the degree of renal damage induced (Mogielnicki et al, 1971). Therefore, an attempt was made to explore the effects in rats of different doses of sodium maleate on glomerular and tubular function. The glomerular permeability was tested by measurements of the urinary clearance of polydisperse ^{125}I-labelled PVP after different doses of sodium maleate had been administered to groups of rats. Fig. 1 shows the clearance of PVP in normal and sodium maleate treated rats. It is evident from the figure that PVP molecules of a size smaller than approximately 25 A had a clearance very similar to that for creatinine, whereas the clearance for molecules larger than approximately 60 A was negligible. The PVP clearance curve obtained in rats having received 150 mg per kg of sodium maleate was within experimental error identical to the normal curve. Rats injected with 400 mg per kg of sodium maleate exhibited on the average a greatly decreased clearance of PVP of all sizes (see Fig. 1). Furthermore, this group of rats had creatin-

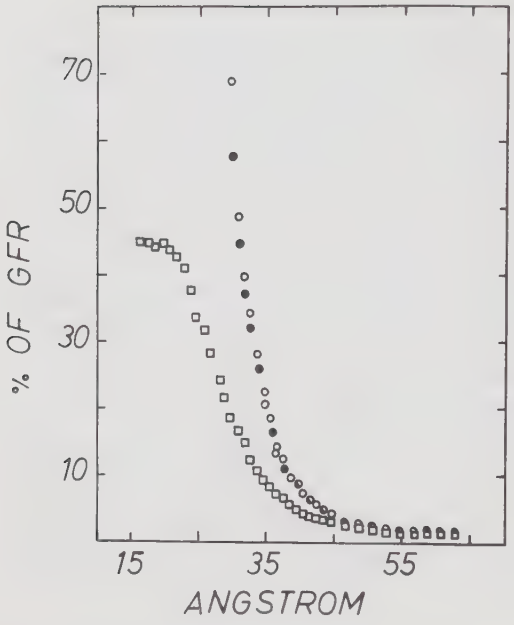

Fig. 1. Relationship between the clearance and molecular size of radioactively labelled polyvinylpyrrolidone (PVP) in normal rats (o), in rats treated with 150 mg per kg of sodium maleate (●), and in rats treated with 400 mg per kg of sodium maleate (□). Each point is the mean of three determinations performed in different rats. The clearance is given as % of endogenous creatinine clearance.

ine clearances of less than 40 % of that obtained for normal rats or rats having received 150 mg sodium maleate per kg.

The effects of sodium maleate on the kidney tubular function were assessed by measurements of the urinary excretion of glucose and by evaluation of the amount and size distribution of excreted proteins. There was a high degree of glucosuria, and the urinary protein pattern observed following gel chromatography was typical for tubular proteinuria, with an increased urinary excretion of low molecular weight proteins in both groups of animals.

Thus, the experiments conducted for evaluation of the glomerular function indicated that administration of 150 mg per kg of sodium maleate did not markedly affect the glomerular permeability. Administration of 400 mg per kg of sodium maleate decreased the excretion of PVP to a considerable extent probably due to the reduced GFR. Both groups of sodium maleate treated animals exhibited, however, obvious signs of tubular damage.

Other methods

Glucosuria was quantitatively assessed employing the total reducing sub-

stance method of Hofmann (1937). Protein was determined by the procedure of Lowry et al. (Lowry, Rosebrough, Farr & Randall, 1951). Serum and urine creatinine were measured by a modification of the Hawk method (Hawk, Oser & Summerson, 1947).

RESULTS

Turnover of β_2-microglobulin in normal, ureter-ligated, and bilaterally nephrectomized rats

The plasma disappearance curves for β_2-microglobulin, labelled with ^{125}I, in normal and ureter-ligated animals are presented in Fig. 2. The curves appear similar and the data calculated from the plasma disappearance curves are in good agreement (Table I). The elimination of β_2-microglobulin from plasma of nephrectomized rats is greatly prolonged compared to normal rats (see Fig. 2). The data calculated from the disappearance curves showed that the Fmr decreased approximately 6-fold in the nephrectomized animals compared to the normal ones (Table I).

The β_2-microglobulin concentration in the renal vein was compared to that of the femoral artery in normal rats. Table II summarizes the result. The concentration in the vein was lower by approximately 10 % compared to the artery, indicating that a substantial extraction of β_2-microglobulin from plasma occurred in the kidney.

Turnover of β_2-microglobulin in sodium maleate treated rats

The plasma disappearance curve of ^{125}I-labelled β_2-microglobulin in rats treated with 150 mg per kg of sodium maleate is depicted in Fig. 3. It can be seen in the figure that the curve obtained is very similar to that found for untreated rats. The data calculated from the disappearance curves clearly demonstrated that sodium maleate at 150 mg per kg had no apparent effect on the disappearance of β_2-microglobulin from plasma (cf. Table I).

Fig. 3 also shows the plasma disappearance curve of β_2-microglobulin for animals treated with 400 mg per kg of sodium maleate. This dose not only caused a tubular damage but also lowered the glomerular filtration rate as measured by the diminished PVP and creatinine clearance (see above). As anticipated, a reduced Fmr for β_2-microglobulin was noted in these animals (Table I).

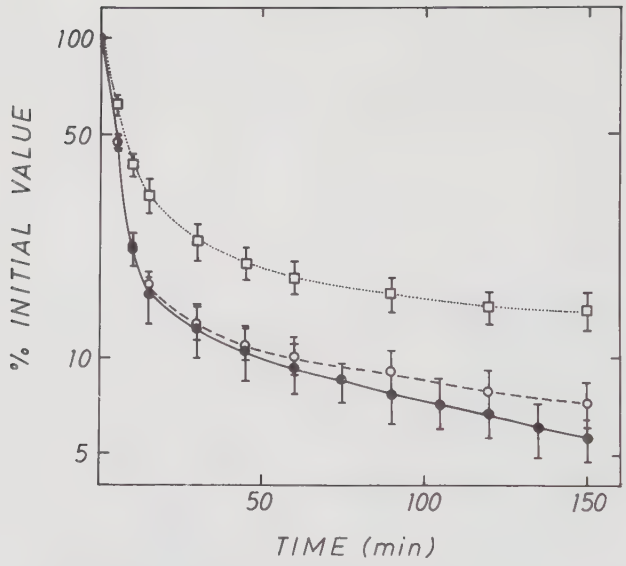

Fig. 2. Plasma disappearance curves for injected radioactively labelled β_2-microglobulin. The points represent mean values and the vertical bars standard deviation for normal rats (7 animals, ●), for ureter-ligated rats (5 animals, o), and for bilaterally nephrectomized rats (4 animals, □).

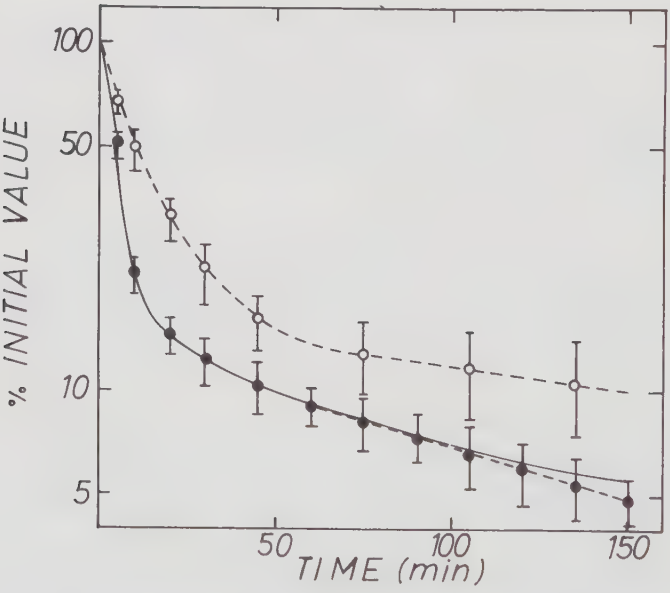

Fig. 3. Plasma disappearance curves for injected radioactively labelled β_2-microglobulin. The points represent mean values and the vertical bars standard deviation for rats treated with 150 mg per kg of sodium maleate (4 rats, ●) and 400 mg per kg of sodium maleate (3 rats, o). The solid line is the disappearance curve for β_2-microglobulin in normal rats and it is included for comparison.

Table I. Turnover data for β_2-microglobulin[a]

Group[b]	Fractional metabolic rate (Fmr)	Biological half-life (BHL)	Intravascular distribution (% IV)
	$hour^{-1}$	hour	%
Normal (7)	2.01 ± 0.20	2.4 ± 0.3	27.5 ± 4.9
Ureter-ligated (5)	1.82 ± 0.16	2.7 ± 0.2	26.5 ± 4.8
Nephrectomized (4)	0.33 ± 0.03	12.3 ± 0.7	28.5 ± 3.2
Sodium maleate treated, 150 mg/kg (4)	2.10 ± 0.35	2.2 ± 0.4	25.2 ± 4.2
Sodium maleate treated, 400 mg/kg (3)	0.86 ± 0.28	6.0 ± 1.1	27.7 ± 3.6
Ureter-ligated and sodium maleate treated, 150 mg/kg (3)	1.27 ± 0.33	4.1 ± 0.8	26.9 ± 4.5

[a] All values represent mean ± SD

[b] Numbers in parentheses denote the number of animals used

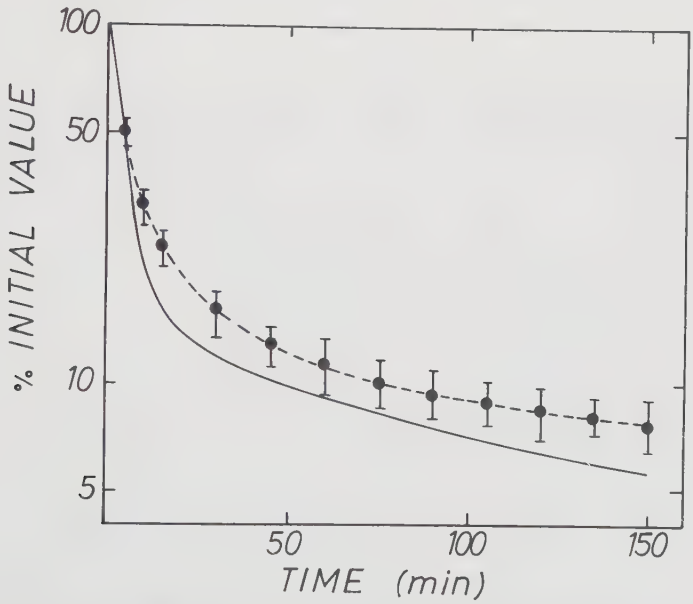

Fig. 4. Plasma disappearance curve for injected radioactively labelled β_2-microglobulin in ureter-ligated rats treated with 150 mg per kg of sodium maleate. The points represent mean values and the vertical bars standard deviation. The data was obtained in 3 animals. The solid curve, representing the disappearance curve for β_2-microglobulin in normal rats, is included for comparison.

Turnover of β_2-microglobulin in sodium maleate treated, ureter-ligated rats

To examine the effect of sodium maleate on the tubular reabsorption of β_2-microglobulin in the kidney, the plasma disappearance curve of β_2-microglobulin in ureter-ligated rats, having received sodium maleate 150 mg per kg was measured. Fig. 4 depicts the result of this experiment. It is evident that ^{125}I-labelled β_2-microglobulin in plasma declined more slowly than in control animals, the Fmr being reduced by approximately 40 % (table I).

Effects of sodium maleate on the urinary excretion of β_2-microglobulin

The excretion of ^{125}I-labelled β_2-microglobulin in urine of normal rats amounted to about 8 % of the intravascular pool per hour. As expected, after sodium maleate treatment the rats excreted considerably elevated amounts of the ^{125}I-labelled protein. Table III summarizes the data on the urinary recovery of intravascularly administered β_2-microglobulin. It is noteworthy that although the rats treated with 400 mg per kg of sodium maleate exhibited a considerable β_2-microglobulinuria, their fractional proteinuric rate was low. This result was, of course, attributable to the low Fmr in this group of rats.

DISCUSSION

Many studies have shown that sodium maleate is a nephrotoxic agent (Mogielnicki et al, 1971; Berliner et al, 1950; Harrison et al, 1954; Rosenberg et al, 1964). Investigations on rats of the morphological changes induced in the kidney by this substance has shown that the major alterations are confined to the tubular cells with flattened brush borders, vacuolation, and thickened basement membranes (Mogielnicki et al, 1971). No changes in the glomeruli or the collecting ducts were, however, observed even when large amounts of sodium maleate were employed. The same histological observations were made on the animals used in this investigation. The morphological changes are thus in good agreement with the abnormal tubular function reported here. In spite of the tubular impairment the glomerular filtration rate (GFR) appeared quite normal after administration of 150 mg of sodium maleate, as measured by PVP or endogenous creatinine clearances. Administration of the larger dose of sodium maleate caused, however, obvious effects on the glomerular filtration rate of both PVP and creatinine in addition to the effects on the tubular function. Since this dose of sodium maleate does not appear to cause any alterations either in the glomeruli,

Table II. Concentrations of β_2-microglobulin in arterial and renal vein plasma

Arterial plasma	Renal vein plasma	
µg/ml	µg/ml	Percentage of arterial conc.
3.52	3.28	93.1
3.64	3.28	90.0
3.45	2.94	85.3
3.81	3.42	89.9
4.62	3.85	83.4
	Mean ± SD	88.3 ± 3.9

Table III. Excretion of β_2-microglobulin in urine of normal and sodium maleate treated rats

Group[a]	Percentage of overall metabolism resulting from proteinuria b	Fractional proteinuric rate (Fpr)	Endogenous fractional catabolic rate (Fcr)
	%	$hour^{-1}$	$hour^{-1}$
Normal (4)	9.2 ± 2.4	0.19	1.83
Sodium maleate treated, 150 mg/kg (4)	21.4 ± 6.9	0.45	1.65
Sodium maleate treated, 400 mg/kg (3)	26.2 ± 9.3	0.08	0.25

[a] Numbers in parentheses denote the number of rats used

[b] Mean ± SD

or in the collecting ducts, as seen by histological examinations (Mogielnicki et al, 1971), the reduced GFR may seem hard to explain. However, kidneys from animals treated with this dose of sodium maleate exhibit considerable luminal plugging with cellular debris. The impairment of the GFR may thus be due to a decreased number of functioning nephrons. This view gains support from the clearance curve recorded for PVP in the present study. It was thus noted that the urinary excretion of PVP of all sizes was diminished which is most easily explained assuming a reduction of the total filtering area of the glomeruli.

The protein used in the present study, β_2-microglobulin, is of human origin. Recently, it has been shown that β_2-microglobulin constitutes one of the two types of transplantation antigen polypeptide chains (Peterson, Rask & Lindblom, 1974). Its concentration in normal biological fluids is very low, which has precluded the isolation of the rat analogue of β_2-microglobulin. Preliminary observations suggest that there is a considerable immunological cross-reactivity between the human and rat proteins. Furthermore, turnover experiments in mice have shown that light immunoglobulin chains have very similar metabolic rates regardless of whether they are of human or murine origin (Wochner et al, 1967). The calculation of the metabolic data presented here assumes that the investigated protein is in a metabolic steady state (Nosslin, 1966). This requirement was, of course, not fullfilled in this study. However, the turnover of β_2-microglobulin was within experimental error identical regardless of the amount injected, varying over a 50-fold range of initial concentration.

The fractional metabolic rate (Fmr) for β_2-microglobulin was high in normal rats. A difference between the β_2-microglobulin concentrations in arterial and renal vein blood was found. It may thus be inferred that the kidney catabolized a large portion of the administered protein. Ureter-ligated rats exhibited the same turnover rate as the normal rats for the β_2-microglobulin. There is a considerable glomerular filtration during the first hours after ureteral ligation (Salomon & Lanza, 1962). It is thus likely that the tubular reabsorption was normal or even enhanced in these animals since the renal catabolism most probably had to compensate for the normal urinary loss. In contrast, the nephrectomized rats had a considerably decreased catabolism of β_2-microglobulin clearly indicating that the kidney is the main catabolic site for this small protein. It is, however, also evident that the renal tissue is not the only tissue for β_2-microglobulin catabolism

since an appreciable degradation occurred also in the absence of kidneys. Assuming that no new ways for β_2-microglobulin catabolism are induced by nephrectomy, the extra-renal degradation amounts to approximately 15 % of the overall metabolism in the normal rat.

Results in accord with the present data were published by Bernier & Conrad (1969). Although direct comparison between this study and that of Bernier & Conrad is precluded, since they did not estimate the actual fractional metabolic rates of the β_2-microglobulin metabolism, the same general conclusions as presented here were reached.

The lower dose of sodium maleate employed (150 mg per kg) did not significantly change the normal Fmr of β_2-microglobulin. The fractional catabolic rate was however decreased but this was compensated by the increased fractional proteinuric rate. This dose of sodium maleate appeared thus to allow a normal glomerular filtration rate of β_2-microglobulin but the protein in the tubular lumen was partially shifted from reabsorption and catabolism to excretion into the urine. The 400 mg per kg dose of sodium maleate affected profoundly the overall metabolism of β_2-microglobulin. The Fmr was reduced by approximately 60 %. Although these animals showed signs of greatly reduced tubular uptake of protein, the fractional proteinuric rate was low due to the low fractional metabolic rate. It is thus obvious that the prolonged biological half life of β_2-microglobulin in these animals was caused by the decreased GFR. This loss of both catabolic and excretory function due to decreased glomerular filtration probably explains the high serum levels and prolonged survival times of small proteins in uremia (Vahlquist et al, 1973; Hansen, Karle, Andersen & Ølgaard, 1972).

If intact tubular cells had a significant transfer of β_2-microglobulin to peritubular blood, damage of the tubular function would result in an increased fractional metabolic rate, since the increased proteinuria would yield an enhanced loss of protein. The fact that the metabolic rate was normal after administration of the lower dose of sodium maleate, strongly suggests that the bulk of reabsorbed protein is degraded within the tubular cells to save amino acids for reutilization. It cannot, however, be completely excluded that some of the protein, trapped in the renal tubuli, may recirculate in the blood since small but significant amounts of protein-bound radioactivity was present in blood even after a substantial time.

According to the above discussion, a partial damage of the tubular function in ureter-ligated rats would decrease the fractional metabolic rate. This should be due to impairment of the renal catabolism together with abolishment of the proteinuria. This was corroborated in ureter-ligated, sodium maleate treated rats. The decrease in total β_2-microglobulin metabolism seen in these animals is explained by the fact that ureteral ligation precluded excretion of the filtered protein which, of course, in these animals should have been substantial due to the tubular derangement.

The present data give strong support to the view that in the normal renal state β_2-microglobulin is easily filtered across the glomerular barrier and most of it is reabsorbed in the renal tubuli. This is in agreement with findings reported for light immunoglobulin chains (Wochner et al, 1967; Mogielnicki et al, 1971). In a study similar to this Mogielnicki et al. (1971) studied the turnover of light chains in normal and sodium maleate treated mice. They concluded that in tubular proteinuria, the clearance of protein from the blood is unchanged but the route of elimination is shifted from tubular catabolism to urinary excretion. The metabolic data for β_2-microglobulin are fully compatible with such a model. Furthermore, tubular proteinuria in man results in a considerably increased excretion of small plasma proteins like β_2-microglobulin (Peterson et al, 1969; Berggård et al, 1968), retinol binding protein (Peterson & Berggård, 1971a), and light immunoglobulin chains (Peterson & Berggård, 1971b) and it is thus highly likely that small proteins like these are handled very similarly by the rat and the human kidney.

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